

LIS009051562B2

(12) United States Patent

Machover et al.

(54) POLYPEPTIDES ISOLATED FROM

BREVIBACTERIUM AURANTIACUM AND

THEIR USE FOR THE TREATMENT OF

CANCER

(75) Inventors: **David Machover**, Villejuif (FR); **Pascal Bonnarme**, Thiverval-grignon (FR)

(73) Assignees: Institut National de la Santé et de la Recherche Médicale (INSERM), Paris (FR); INRA (Institut National de la Recherche Agronomique), Paris Cedex (FR); UNIVERSITE PARIS SUD-PARIS XI, Orsay Cedex (FR)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35

U.S.C. 154(b) by 0 days.

(21) Appl. No.: 14/126,253

(22) PCT Filed: Jun. 15, 2012

(86) PCT No.: PCT/EP2012/061497

§ 371 (c)(1),

(2), (4) Date: Feb. 4, 2014

(87) PCT Pub. No.: WO2012/172074PCT Pub. Date: Dec. 20, 2012

(65) Prior Publication Data

US 2014/0140978 A1 May 22, 2014

Related U.S. Application Data

(60) Provisional application No. 61/504,363, filed on Jul. 5, 2011.

(30) Foreign Application Priority Data

(51) Int. Cl. C07K 14/345 (2006.01) A61K 38/00 (2006.01) C12N 9/88 (2006.01) C12N 9/14 (2006.01) C07K 14/435 (2006.01)

(10) Patent No.:

US 9,051,562 B2

(45) **Date of Patent:**

Jun. 9, 2015

(56) References Cited

FOREIGN PATENT DOCUMENTS

WO 00/29589 A1 5/2000

OTHER PUBLICATIONS

El-Sayed, 2010, Microbial L-methioninase: production, molecular characterization, and therapeutic applications, Appl. Microbiol. Biotechnol. 86: 445-467.*

Sato et al., 2009, Methionine Gamma-Lyase: The Unique Reaction Mechanism, Physiological Roles, and Therapeutic Applications Against Infectious Diseases and Cancers, Life, 61(11): 1019-1028.* Hanniffy et al., 2009, Heterologous Production of Methionine—Lyase from *Brevibacterium linens* in *Lactococcus lactis* and Formation of Volatile Sulfur Compounds, Applied and Environmental Microbiology, 75(8): 2326-2332.*

Van Rite et al., 2011, Enzyme prodrug therapy designed to target L-methioninase to the tumor vasculature, Cancer Letters, 301: 177-184 *

Amarita et al., 2004, Identification and Functional Analysis of the Gene Encoding Methionine-_-Lyase in *Brevibacterium linens*, Applied and Environmental Microbiology, 70(12): 7348-7354.*

Dias et al., 1998, Purification and Characterization of L-Methionine _-Lyase from *Brevibacterium linens* BL2, Applied and Environmental Microbiology, 64(9): 3327-3331.*

Cholet et al., 2007, Transcriptional analysis of L-methionine catabolism in *Brevibacterium linens* ATCC9175, Appl. Microbiol. Biotechnol., 74: 1320-1332.*

Database Embl, "Brevibacterium linens L-mehionine-gamma-lyase (mgl) gene, complete cds.", XP002664727, Dec. 10, 2004, retrieved from EBI accession no. EMBL:AY622198, 1 page.

Tan Y, et al., "Polyethylene Glycol Conjugation of Recombinant Methioninase for Cancer Therapy", Protein Expression and Purification, Feb. 1, 1998, pp. 45-52, vol. 12, No. 1, Academic Press, San Diego, CA

Machover David, et al., "Cytotoxic synergism of methioninase in combination with 5-fluorouracil and folinic acid", Biochemical Pharmacology, Apr. 1, 2001, pp. 867-876, vol. 61, No. 7.

Ashraf S El-Sayed, "Microbial I-methioninase: production, molecular characterization, and therapeutic applications", Applied Microbiology and Biotechnology, Feb. 10, 2010, pp. 445-467, vol. 86, No. 2, Springer, Berlin, DE.

Tan Y, et al., "Serum Methionine depletion without side effects by methioninase in metastatic breast cancer patients", Anticancer Research, Nov. 1, 1996, pp. 3937-3942, vol. 16, No. 6C, International Institute of Anticancer Research, GR.

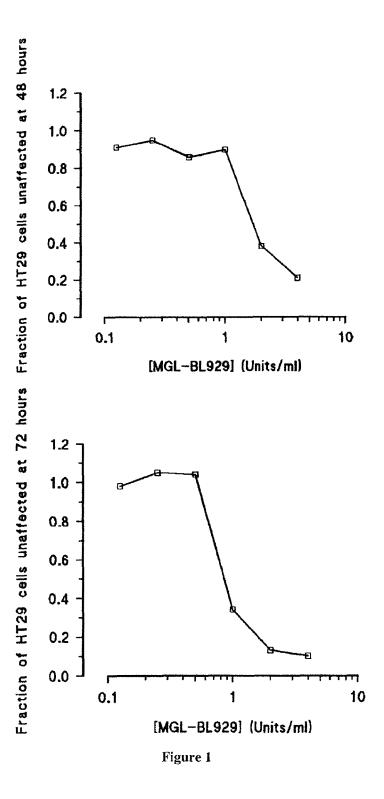
* cited by examiner

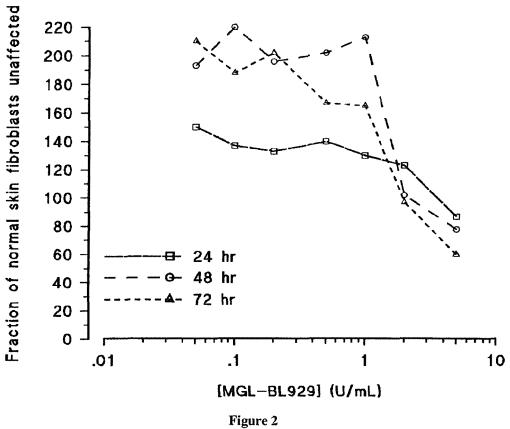
Primary Examiner — Amber D Steele (74) Attorney, Agent, or Firm — Whitham Curtis Christofferson & Cook, PC

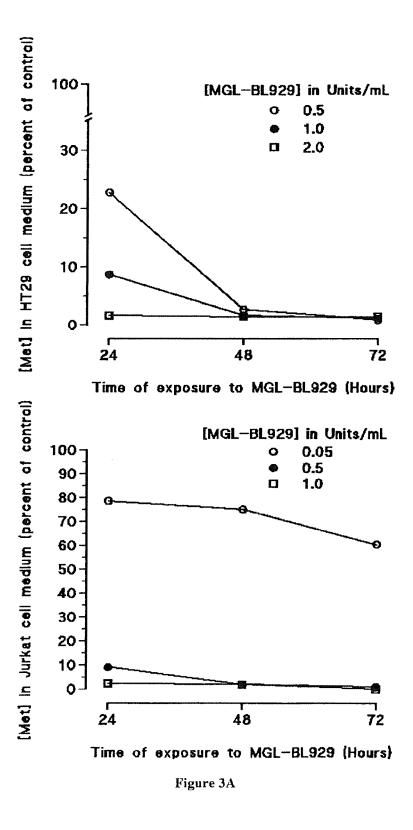
(57) ABSTRACT

The present invention relates to methods and pharmaceutical compositions for treating cancer. More specifically, the invention relates to a polypeptide isolated from *Brevibacterium aurantiacum* that shows methionine gamma-lyase and homocysteinase activities. The present invention also relates to the use of such a polypeptide for the treatment of cancer.

16 Claims, 6 Drawing Sheets







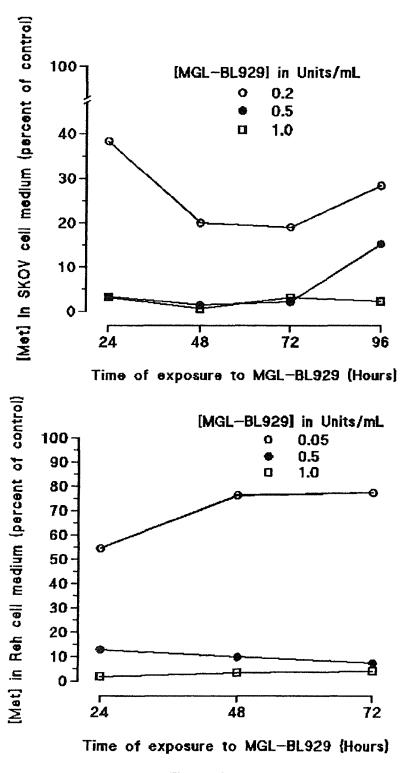


Figure 3B

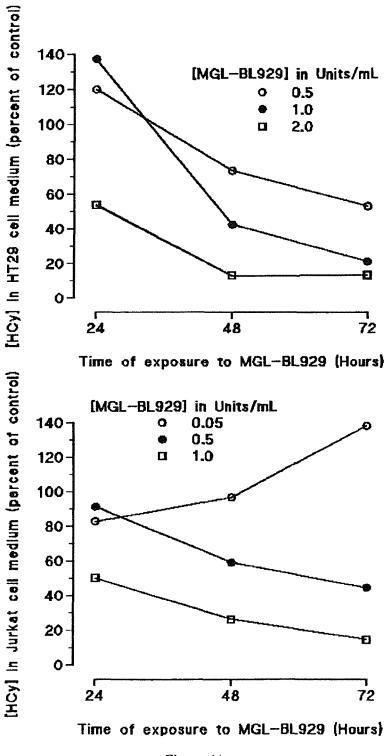
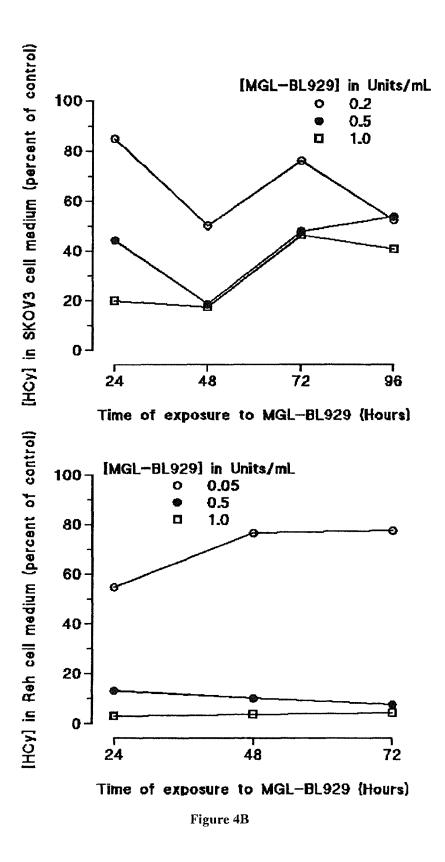


Figure 4A



POLYPEPTIDES ISOLATED FROM BREVIBACTERIUM AURANTIACUM AND THEIR USE FOR THE TREATMENT OF CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to International Application No. PCT/EP2012/061497, filed Jun. 15, 2012, which claims priority to European Application No. 11305748.3, filed Jun. 15, 2011 and U.S. Application No. 61/504,363, filed Jul. 5, 2011, the complete contents of which are herein incorporated by reference.

FIELD OF THE INVENTION

The invention relates to polypdetides isolated from $Brevi-bacterium\ aurantiacum$ that shows methionine gamma-lyase and homocysteinase activities and their use for the treatment of cancer.

BACKGROUND OF THE INVENTION

Cytotoxicity of cancer cells grown under methionine deprivation has been well established. Depletion of methionine induces death of cancer cells, whereas normal cells are much more resistant. The reasons for this relative selectivity are unknown. Normal and tumor cells can synthesize methionine 30 provided sufficient folate, cobalamin, and homocysteine are supplied. However, most tumor cells require larger amounts of methionine than what they can synthesize, and, in the absence of an exogenous supply, they experience growth inhibition or die.

Depletion of methionine in vitro and in vivo can be achieved through the action of methionine gamma-lyase (L-methionine- α -deamino- γ -mercaptoethane lyase; MGL) that catalyses irreversibly the α,γ -elimination of L-methionine resulting in production of methanethiol, α -ketobutyrate, 40 and ammonia. Various MGLs have been produced by purification from several microorganisms or by recombination of genes encoding for the enzyme originating from various bacterial species and protozoa. Most studies on the antitumor action of methionine depletion have used the MGL from the 45 potentially pathogen pseudomonade, *Pseudomonas putida* (Pp-MGL).

Methionine depletion obtained with Pp-MGL enhances the cytotoxic action of the fluoropyrimidine 5-fluorouracil (FUra), an agent currently used for the treatment of various 50 types of human cancer (Machover et al., 2001; Machover et al., 2002). Potentiation is due to modifications of the intracellular pools of reduced folates induced by methionine depletion and, possibly, through changes in the expression of cellular mechanisms favouring cell death, which may be related 55 to DNA demethylation (Machover et al., 2001; Machover et al., 2002). Other investigators have demonstrated potentiation of FUra and cisplatin in tumor-bearing mice by simultaneous administration of Pp-MGL.

Animal experiments have been performed with the aim to 60 introduce recombinant Pp-MGL in the clinics. However, lethal anaphylactic shock syndromes have been observed when monkeys were re challenged with the protein, which has prevented further development of the native recombinant Pp-MGL. Attempts at reduction of the immunogenicity of the 65 protein through pegylation have not succeeded at the present time.

2

This pre clinical data believed that the prohibitive immunogenicity of the P.p.MGL, and possibly that of other putative and well characterized MGLs described thus far, which derive all from various micro organisms that are potential pathogens for humans (i.e., originating from the bacteria Aeromonas sp., Citrobacter freundii, Porphyromonas gingivalis, and Treponema denticola, and the protozoa Trichomonas vaginalis, and Entamoeba histolytica), could not allow intra venous administration of the enzyme required for sustained methionine depletion in plasma under safe conditions.

However, there is a need to develop new drugs against cancer. In this way, it has been suggested that characterisation of new therapeutic targets inhibiting tumor cell growth may be highly desirable. There is thus a need in the art for methionine gamma-lyase derived from a non pathogenic micro organism abundantly present in food, this MGL may benefit from oral immune tolerance allowing its administration into the blood stream in a subject in need thereof for treatment of cancer.

SUMMARY OF THE INVENTION

The present invention relates to methods and pharmaceutical compositions for treating cancer. More specifically, the invention relates to *Brevibacterium aurantiacum* methionine gamma-lyase and their use for the treatment of cancer.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the development of methionine gamma-lyase from *Brevibacterium aurantiacum* (ATCC 9175; formerly *Brevibacterium linens*).

The inventors have studied gene expression by transcriptome analysis in the presence of various sulfur-containing amino acids in *B. aurantiacum* (ATCC 9175 a cheese ripening micro organism characterized by aroma and pigment production, and by its property of inhibiting the growth of food pathogens. Surprisingly, these studies led to the identification of the sequence of a new putative methionine gamma-lyase (MGL), greatly different from that previously described in the same micro organism (Amarita et al., 2004).

The inventors have transformed an *E. coli* production strain with an expression plasmid containing the optimized gene of the putative MGL from *B. aurantiacum*. The recombinant enzyme MGL-BL929 produced has shown specific activity for methionine between 4.33 U/mg and 7.21 U/mg, i.e., values near those found for the enzyme derived from *P. putida*. The inventors have demonstrated that MGL-BL929 produced possesses in addition of methionine gamma-lyase activity, homocysteinase activity. The inventors have also demonstrated that MGL-BL929

depletes methionine and homocysteine from tumor-cell culture media for long periods,

is stable under tumor cell culture conditions as well as in human serum in vitro,

possesses high cytotoxic activity against various human tumor cells growing in culture,

does not affect growth of normal cells at concentrations that are highly cytotoxic for cancer cells,

potentiates (modulates) the cytotoxic activity of cytostatic agents, and

circumvents prior cell resistance to cytostatics

DEFINITIONS

The term "methionine gamma-lyase" or "MGL" has its general meaning in the art and refers to L-methionine- α -

deamino- γ -mercaptoethane lyase, the enzyme that catalyses irreversibly the α , γ -elimination of L-methionine resulting in production of methanethiol, α -ketobutyrate, and ammonia.

The term "homocysteinase activity" has its general meaning in the art and refers to L-homocysteine lyase activity.

The term "Brevibacterium linens" also encompasses three other species, namely Brevibacterium aurantiacum, Brevibacterium antiquum and Brevibacterium permense as described by Gavrish et al. 2004. Strain ATCC 9175 was deposited as B. linens ATCC9175 at the American Type Culture Collection and recently renamed as B. aurantiacum ATCC9175 as proposed by Gavrish et al. (2004).

A "coding sequence" or a sequence "encoding" an expression product, such as a RNA, polypeptide, protein, or enzyme, is a nucleotide sequence that, when expressed, 15 results in the production of that RNA, polypeptide, protein, or enzyme, i.e., the nucleotide sequence encodes an amino acid sequence for that polypeptide, protein or enzyme. A coding sequence for a protein may include a start codon (usually ATG) and a stop codon.

By "purified" and "isolated" it is meant, when referring to a polypeptide (i.e. the methionine gamma-lyase of the invention) or a nucleotide sequence, that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. The term "purified" as used 25 herein preferably means at least 75% by weight, more preferably at least 85% by weight, still preferably at least 95% by weight, and most preferably at least 98% by weight, of biological macromolecules of the same type are present. An "isolated" nucleic acid molecule which encodes a particular polypeptide refers to a nucleic acid molecule which is substantially free of other nucleic acid molecules that do not encode the subject polypeptide; however, the molecule may include some additional bases or moieties which do not deleteriously affect the basic characteristics of the composition. 35

The term "PEG" encompasses any polyethylene glycol molecule, without regard to size or to modification at an end of the PEG, and can be represented by the formula X—O (CH₂CH₂O)n-OH, where n is an integer between 20 and 2300, and X is H or a terminal modification, e.g., alkyl.

By "pegylation" is meant the process by which polyethylene glycol (PEG) chains are attached to polypeptides. The term "pegylated polypeptide" denotes a polypeptide that comprises at least one PEG group covalently conjugated to said polypeptide.

As used herein, the term "subject" denotes a mammal, such as a rodent, a feline, a canine, and a primate. Preferably a subject according to the invention is a human.

Methionine Gamma-Lyase Polypeptides

The invention thus provides an isolated, synthetic or recombinant methionine gamma-lyase polypeptide comprising an amino acid sequence as set forth by SEQ ID NO: 1 or a function-conservative variant thereof.

The present invention thus encompasses function-conservative variants of the methionine gamma-lyase polypeptides as set forth by SEQ ID NO:1. The function-conservative variants may result from modifications and changes that may be made in the structure of the polypeptides of SEQ ID NO:1 60 (and in the DNA sequences encoding it), and still obtain a functional molecule with desirable characteristics (MGL and homocysteinase).

Accordingly, "function-conservative variants" are those in which a given amino acid residue in a protein or enzyme has 65 been changed without altering the overall conformation and function of the polypeptide, including, but not limited to,

4

replacement of an amino acid with one having similar properties (such as, for example, polarity, hydrogen bonding potential, acidic, basic, hydrophobic, aromatic, and the like). Amino acids other than those indicated as conserved may differ in a protein so that the percent protein or amino acid sequence similarity between any two proteins of similar function may vary and may be, for example, from 70% to 99% as determined according to an alignment scheme such as by the Cluster Method, wherein similarity is based on the MEGA-LIGN algorithm. A "function-conservative variant" also includes a polypeptide which has at least 60% amino acid identity as determined by BLAST or FASTA algorithms, preferably at least 75%, more preferably at least 85%, still preferably at least 90%, and even more preferably at least 95%, and which has the same or substantially similar properties or functions as the native or parent protein to which it is compared. Two amino acid sequences are "substantially homologous" or "substantially similar" when greater than 80%, preferably greater than 85%, preferably greater than 90% of the amino acids are identical, or greater than about 90%, preferably grater than 95%, are similar (functionally identical). Preferably, the similar or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wis.) pileup program, or any of sequence comparison algorithms such as BLAST, FASTA, etc.

The amino acid changes may be achieved by changing codons in the DNA sequence, according to Table 1.

TABLE 1

| Amin | o acids | | Codons | | | | |
|---------------|---------|---|------------------------------|--|--|--|--|
| Alanine | Ala | A | GCA, GCC, GCG, GCU | | | | |
| Cysteine | Cys | С | UGC, UGU | | | | |
| Aspartic Acid | Asp | D | GAC, GAU | | | | |
| Glutamic acid | Glu | E | GAA, GAG | | | | |
| Phenylalanine | Phe | F | UUC, UUU | | | | |
| Glycine | Gly | G | GGA, GGC, GGG, GGU | | | | |
| Histidine | His | Η | CAC, CAU | | | | |
| Isoleucine | Ile | Ι | AUA, AUC, AUU | | | | |
| Lysine | Lys | K | AAA, AAG | | | | |
| Leucine | Leu | L | UUA, UUG, CUA, CUC, CUG, CUU | | | | |
| Methionine | Met | M | AUG | | | | |
| Asparagine | Asn | N | AAC, AAU | | | | |
| Proline | Pro | P | CCA, CCC, CCG, CCU | | | | |
| Glutamine | Gln | Q | CAA, CAG | | | | |
| Arginine | Arg | R | AGA, AGG, CGA, CGC, CGG, CGU | | | | |
| Serine | Ser | S | AGC, AGU, UCA, UCC, UCG, UCU | | | | |
| Threonine | Thr | T | ACA, ACC, ACG, ACU | | | | |
| Valine | Val | V | GUA, GUC, GUG, GUU | | | | |
| Tryptophan | Trp | W | UGG | | | | |
| Tyrosine | Tyr | Y | UAU | | | | |

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of methionine gamma-lyase capability or homocystein-ase capability. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid substitutions can be made in a protein sequence, and, of course, in its DNA encoding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated that various changes may be made in the polypeptide sequences of the invention, or corresponding DNA sequences which encode said polypeptides, without appreciable loss of their biological activity.

Said methionine gamma-lyase activity and homocysteinase activity can be assessed by different techniques wellknown in the art as described hereinafter.

In making the changes in the amino sequences of polypeptide, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art. It is accepted that the relative hydro- 5 pathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic 10 index on the basis of their hydrophobicity and charge characteristics these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophane (-0.9); tyrosine (-1.3); 15 proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic 20 index or score and still result in a protein with similar biological activity, i.e. still obtain a biological functionally equivalent protein.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid 25 side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; 30 serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

The methionine gamma-lyase activity and homocysteinase activity of the function-conservative variants may be assessed according any method of assaying methionine gamma-lyase 35 activity and homocysteinase activity known in the art, such as for instance the assays referred in the instant application.

The polypeptides of the invention may be produced by any technique known per se in the art, such as, without limitation, any chemical, biological, genetic or enzymatic technique, 40 either alone or in combination.

Knowing the amino acid sequence of the desired sequence, one skilled in the art can readily produce said polypeptides, by standard techniques for production of polypeptides. For instance, they can be synthesized using well-known solid 45 phase method, preferably using a commercially available peptide synthesis apparatus (such as that made by Applied Biosystems, Foster City, Calif.) and following the manufacturer's instructions.

Alternatively, the polypeptides of the invention can be 50 synthesized by recombinant DNA techniques as is now well-known in the art. For example, these fragments can be obtained as DNA expression products after incorporation of DNA sequences encoding the desired (poly)peptide into expression vectors and introduction of such vectors into suitable eukaryotic or prokaryotic hosts that will express the desired polypeptide, from which they can be later isolated using well-known techniques.

Polypeptides of the invention can be use in an isolated (e.g., purified) form or contained in a vector, such as a membrane or 60 lipid vesicle (e.g. a liposome).

In specific embodiments, it is contemplated that polypeptides according to the invention may be modified in order to improve their therapeutic efficacy. Such modification of therapeutic compounds may be used to decrease toxicity, 65 increase circulatory time, or modify biodistribution. For example, the toxicity of potentially important therapeutic

6

compounds can be decreased significantly by combination with a variety of drug carrier vehicles that modify biodistribution

A strategy for improving drug viability is the utilization of water-soluble polymers. Various water-soluble polymers have been shown to modify biodistribution, improve the mode of cellular uptake, change the permeability through physiological barriers; and modify the rate of clearance from the body. To achieve either a targeting or sustained-release effect, water-soluble polymers have been synthesized that contain drug moieties as terminal groups, as part of the backbone, or as pendent groups on the polymer chain.

For example, Pegylation is a well established and validated approach for the modification of a range of polypeptides (Chapman, 2002). The benefits include among others: (a) markedly improved circulating half-lives in vivo due to either evasion of renal clearance as a result of the polymer increasing the apparent size of the molecule to above the glomerular filtration limit, and/or through evasion of cellular clearance mechanisms; (b) reduced antigenicity and immunogenicity of the molecule to which PEG is attached; (c) improved pharmacokinetics; (d) enhanced proteolytic resistance of the conjugated protein (Cunningham-Rundles et. al., 1992); and (e) improved thermal and mechanical stability of the PEGylated polypeptide.

Therefore, advantageously, the polypeptides of the invention may be covalently linked with one or more polyethylene glycol (PEG) group(s).

Accordingly, one aspect of the invention provides modified polypeptides, wherein the modification comprises a single polyethylene glycol group covalently conjugated to the polypeptide. Other aspects provide modified polypeptides covalently conjugated to one, two, three, or more polyethylene glycol groups. The one or more PEG may have a molecular weight ranging from about 1 kDa to about 100 kDa, and will preferably have a molecular weight ranging from about 10 to about 60 kDa or about 10 to about 40 kDa. One skilled in the art can select a suitable molecular mass for PEG, based on how the pegylated polypeptide will be used therapeutically by considering different factors including desired dosage, circulation time, resistance to proteolysis, immunogenicity,

In one embodiment, the PEG of the invention terminates on one end with hydroxy or methoxy, i.e., X is H or CH_3 ("methoxy PEG"). In addition, such a PEG can consist of one or more PEG side-chains which are linked together. PEGs with more than one PEG chain are called branched PEGs. Branched PEGs can be prepared, for example, by the addition of polyethylene oxide to various polyols, including glycerol, pentaerythriol, and sorbitol. For example, a four-armed branched PEG can be prepared from pentaerythriol and ethylene oxide. One form of PEGs includes two PEG side-chains (PEG2) linked via the primary amino groups of a lysine (Monfardini, et al., 1995).

To effect covalent attachment of PEG groups to the polypeptide, the hydroxyl end groups of the polymer molecule must be provided in activated form, i.e. with reactive functional groups (examples of which include primary amino groups, hydrazide (HZ), thiol, succinate (SUC), succinimidyl succinate (SSA), succinimidyl succinate (SSA), succinimidyl proprionate (SPA), succinimidyl carboxymethylate (SCM), benzotriazole carbonate (BTC), N-hydroxysuccinimide (NHS), aldehyde, nitrophenylcarbonate (NPC), and tresylate (TRES)). Suitable activated polymer molecules are commercially available, e.g. from Shearwater Polymers, Inc., Huntsville, Ala., USA, or from PolyMASC Pharmaceuticals plc, UK. Alternatively, the polymer molecules can be acti-

vated by conventional methods known in the art, e.g. as disclosed in WO 90/13540. Specific examples of activated linear or branched polymer molecules for use in the present invention are described in the Shearwater Polymers, Inc. 1997 and 2000 Catalogs (Functionalized Biocompatible Polymers for 5 Research and pharmaceuticals, Polyethylene Glycol and Derivatives, incorporated herein by reference). Specific examples of activated PEG polymers include the following linear PEGs: NHS-PEG (e.g. SPA-PEG, SSPA-PEG, SBA-PEG, SS-PEG, SSA-PEG, SC-PEG, SG-PEG, and SCM-PEG), and NOR-PEG, BTC-PEG, EPDX-PEG, NCO-PEG, NPC-PEG, CDI-PEG, ALD-PEG, TRES-PEG, VS-PEG, IODO-PEG, and MAL-PEG, and branched PEGs such as PEG2-NHS.

The conjugation of the polypeptide and the activated polymer molecules is conducted by use of any conventional method. Conventional methods are known to the skilled artisan. The skilled person will be aware that the activation method and/or conjugation chemistry to be used depends on the attachment group(s) of the polypeptides as well as the functional groups of the PEG molecule (e.g., being amine, hydroxyl, carboxyl, aldehyde, ketone, sulfhydryl, succinimidyl, maleimide, vinylsulfone or haloacetate).

In one embodiment, polypeptides are conjugated with PEGs at amino acid D and E (for COOH), T, Y and S (for OH), 25 K (for NH₂), C (for SH if at least one cysteine is conserved) or/and Q and N (for the amide function).

In one embodiment, additional sites for PEGylation can be introduced by site-directed mutagenesis by introducing one or more lysine residues. For instance, one or more arginine ³⁰ residues may be mutated to a lysine residue. In another embodiment, additional PEGylation sites are chemically introduced by modifying amino acids on polypeptides of the invention.

In one embodiment, PEGs are conjugated to the polypep- ³⁵ tide through a linker. Suitable linkers are well known to the skilled person. A preferred example is cyanuric chloride (Abuchowski et al., 1977; U.S. Pat. No. 4,179,337).

Conventional separation and purification techniques known in the art can be used to purify pegylated polypeptides 40 of the invention, such as size exclusion (e.g. gel filtration) and ion exchange chromatography. Products may also be separated using SDS-PAGE.

In one embodiment, the pegylated polypeptides provided by the invention have a serum half-life in vivo at least 50%, 45 75%, 100 %, 150 % or 200 % greater than that of an unmodified polypeptide.

In another embodiment the polypeptides of the invention are covalently coupled to a tumor targeting agent as well known in the art.

Non limiting examples include but are not limited to antibodies directed against the EDB domain of fibronectin, antibodies or agents binding Vascular endothelial growth factor receptor 2, antibodies or molecules binding fibroblast growth factor receptor-1, antibodies or agents that interact with 55 CD31, antibodies or agents interacting with tumor lymphatic endothelium (Podoplanin, Lyve-1), or antibodies or agents binding to $\alpha V\beta 3$ integrin such as RGD peptides, or antibodies or agents interacting with tumor membrane-bound and intracellular targets.

Nucleic Acids, Vectors and Recombinant Host Cells

Another object of the invention relates to an isolated, synthetic or recombinant nucleic acid encoding for a methionine 65 gamma-lyase polypeptide according to the invention. In a particular embodiment, said nucleic acid comprises a

8

sequence as set forth by SEQ ID NO: 2. In another particular embodiment, said nucleic acid comprises a sequence as set forth by SEQ ID NO: 3.

Typically, said nucleic acid is a DNA or RNA molecule, which may be included in any suitable vector, such as a plasmid, cosmid, episome, artificial chromosome, phage or a viral vector. The terms "vector", "cloning vector" and "expression vector" mean the vehicle by which a DNA or RNA sequence (e.g. a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (e.g. transcription and translation) of the introduced sequence.

So, another object of the invention relates to a vector comprising a nucleic acid of the invention.

Such vectors may comprise regulatory elements, such as a promoter, enhancer, terminator and the like, to cause or direct expression of said polypeptide upon administration to a subject. The vectors may further comprise one or several origins of replication and/or selectable markers. The promoter region may be homologous or heterologous with respect to the coding sequence, and provide for ubiquitous, constitutive, regulated and/or tissue specific expression, in any appropriate host cell, including for in vivo use. Examples of promoters include bacterial promoters (T7, pTAC, Trp promoter, etc.), viral promoters (LTR, TK, CMV-IE, etc.), mammalian gene promoters (albumin, PGK, etc), and the like.

Examples of plasmids include replicating plasmids comprising an origin of replication, or integrative plasmids, such as for instance pUC, pcDNA, pBR, and the like. Examples of viral vector include adenoviral, retroviral, herpes virus and AAV vectors. Such recombinant viruses may be produced by techniques known in the art, such as by transfecting packaging cells or by transient transfection with helper plasmids or viruses. Typical examples of virus packaging cells include PA317 cells, PsiCR1P cells, GPenv+ cells, 293 cells, etc. Detailed protocols for producing such replication-defective recombinant viruses may be found for instance in WO 95/14785, WO 96/22378, U.S. Pat. No. 5,882,877, U.S. Pat. No. 6,013,516, U.S. Pat. No. 4,861,719, U.S. Pat. No. 5,278, 056 and WO 94/19478.

Another object of the present invention relates to a cell which has been transfected, infected or transformed by a nucleic acid and/or a vector according to the invention. The term "transformation" means the introduction of a "foreign" 45 (i.e. extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. A host cell that receives and expresses introduced DNA or RNA has been "transformed".

The nucleic acids of the invention may be used to produce a recombinant polypeptide of the invention in a suitable expression system. The term "expression system" means a host cell and compatible vector under suitable conditions, e.g. for the expression of a protein coded for by foreign DNA carried by the vector and introduced to the host cell.

Common expression systems include *E. coli* host cells and plasmid vectors, insect host cells and Baculovirus vectors, and mammalian host cells and vectors. Other examples of host cells include, without limitation, prokaryotic cells (such as bacteria) and eukaryotic cells (such as yeast cells, mammalian cells, insect cells, plant cells, etc.). Specific examples include *E. coli, Kluyveromyces* or *Saccharomyces* yeasts, mammalian cell lines (e.g., Vero cells, CHO cells, 3T3 cells, COS cells, etc.) as well as primary or established mammalian cell cultures (e.g., produced from lymphoblasts, fibroblasts, embryonic cells, epithelial cells, nervous cells, adipocytes,

etc.). More particularly, the invention contemplates vascular or endothelial cells thereof or derived thereof, such as human umbilical vein endothelial (HUVEC) or progenitor endothelial cells (PEC).

The present invention also relates to a method for producing a recombinant host cell expressing a methionine gammalyase polypeptide according to the invention, said method comprising the steps consisting of: (i) introducing in vitro or ex vivo a recombinant nucleic acid or a vector as described above into a competent host cell, (ii) culturing in vitro or ex vivo the recombinant host cell obtained and (iii), optionally, selecting the cells which express and/or secrete said methionine gamma-lyase polypeptide. Such recombinant host cells can be used for the production of methionine gamma-lyase polypeptides according to the present invention, as previously described.

The invention further relates to a method of producing a methionine gamma-lyase polypeptide according to the invention, which method comprises the steps consisting of: (i) 20 culturing a transformed host cell according to the invention under conditions suitable to allow expression of said methionine gamma-lyase polypeptide; and (ii) recovering the expressed polypeptide.

Therapeutic Methods and Uses

In one embodiment, the invention provides a method for treating cancer comprising administering a subject in need thereof with a therapeutically effective amount of a polypeptide or nucleic acid of the invention.

In the context of the invention, the term "treating" or "treatment", as used herein, means reversing, alleviating, inhibiting the progress of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such 35 disorder or condition.

According to the invention, the term "subject" or "subject in need thereof", is intended for a human or non-human mammal affected or likely to be affected with a cancer.

By a "therapeutically effective amount" of the polypeptide 40 of the invention is meant a sufficient amount of the polypeptide to treat a cancer, (for example, to limit tumor growth or to slow or block tumor metastasis) at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the polypeptides 45 and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the 50 disorder; activity of the specific polypeptide employed; the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific polypeptide employed; the duration of the treatment; 55 drugs used in combination or coincidental with the specific polypeptide employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to 60 gradually increase the dosage until the desired effect is achieved.

In a particular embodiment the polypeptide or nucleic acid of the invention may be administered concomitantly with one or more agents required for function of the polypeptide, such as enzyme cofactors. For example, said enzyme cofactor is a pyridoxal phosphate.

10

In a particular embodiment the polypeptide or nucleic acid of the invention may be administered sequentially or concomitantly with one or more chemotherapeutic or radiotherapeutic agents.

In one embodiment said chemotherapeutic or radiotherapeutic agents are a therapeutic active agent used as anticancer agent. For example, said anticancer agents include but are not limited to fludarabine, gemcitabine, capecitabine, methotrexate, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, platinum complexes such as cisplatin, carboplatin and oxaliplatin, mitomycin, dacarbazine, procarbazine, epipodophyllotoxins such as etoposide and teniposide, camptothecins such as irinotecan and topotecan, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, L-asparaginase, doxorubicin, epirubicin, 5-fluorouracil and 5-fluorouracil combined with leucovorin, taxanes such as docetaxel and paclitaxel, levamisole, estramustine, nitrogen mustards, nitrosoureas such as carmustine and lomustine, vinca alkaloids such as vinblastine, vincristine, vindesine and vinorelbine, imatinib mesylate, hexamethylmelamine, kinase inhibitors, phosphatase inhibitors, ATPase inhibitors, tyrphostins, protease inhibitors, inhibitors herbimycin A, genistein, erbstatin, and lavendustin A. In one embodiment, additional 25 anticancer agents may be selected from but are not limited to, one or a combination of the following class of agents: alkylating agents, plant alkaloids, DNA topoisomerase inhibitors, anti-folates, pyrimidine analogs, purine analogs, DNA antimetabolites, taxanes, podophyllotoxins, hormonal therapies, retinoids, photosensitizers or photodynamic therapies, angiogenesis inhibitors, antimitotic agents, isoprenylation inhibitors, cell cycle inhibitors, actinomycin, bleomycin, anthracyclines, MDR inhibitors and Ca²⁺ ATPase inhibitors.

Additional anticancer agents may be selected from, but are not limited to, cytokines, chemokines, growth factors, growth inhibitory factors, hormones, soluble receptors, decoy receptors, monoclonal or polyclonal antibodies, mono-specific, bi-specific or multi-specific antibodies, monobodies, polybodies

Further therapeutic active agent can be an antiemetic agent. Suitable antiemetic agents include, but are not limited to, metoclopramide, domperidone, prochlorperazine, promethazine, chlorpromazine, trimethobenzamide, ondansetron, granisetron, hydroxyzine, acetylleucine, alizapride, azasetron, benzquinamide, bietanautine, bromopride, buclizine, clebopride, cyclizine, dimenhydrinate, diphenidol, dolasetron, meclizine, methallatal, metopimazine, nabilone, pipamazine, scopolamine, sulpiride, tetrahydrocannabinols, thiethylperazine, thioproperazine and tropisetron. In a preferred embodiment, the antiemetic agent is granisetron or ondansetron.

In another embodiment, the further therapeutic active agent can be an hematopoietic colony stimulating factor. Suitable hematopoietic colony stimulating factors include, but are not limited to, filgrastim, sargramostim, molgramostim and epoietin alpha.

In still another embodiment, the other therapeutic active agent can be an opioid or non-opioid analgesic agent. Suitable opioid analgesic agents include, but are not limited to, morphine, heroin, hydromorphone, hydrocodone, oxymorphone, oxycodone, metopon, apomorphine, buprenorphine, meperidine, loperamide, ethoheptazine, betaprodine, diphenoxylate, fentanyl, sufentanil, alfentanil, remifentanil, levorphanol, dextromethorphan, phenazone, pemazocine, cyclazocine, methadone, isomethadone and propoxyphene. Suitable non-opioid analgesic agents include, but are not limited to, aspirin, celecoxib, rofecoxib, diclofenac,

diflunisal, etodolac, fenoprofen, flurbiprofen, ibuprofen, ketoprofen, indomethacin, ketorolac, meclofenamate, mefenamic acid, nabumetone, naproxen, piroxicam and sulindac.

In yet another embodiment, the further therapeutic active agent can be an anxiolytic agent. Suitable anxiolytic agents include, but are not limited to, buspirone, and benzodiazepines such as diazepam, lorazepam, oxazapam, clorazepate, clonazepam, chlordiazepoxide and alprazolam.

The term "radiotherapeutic agent" as used herein, is intended to refer to any radiotherapeutic agent known to one of skill in the art to be effective to treat or ameliorate cancer, without limitation. For instance, the radiotherapeutic agent can be an agent such as those administered in brachytherapy or radionuclide therapy. Such methods can optionally further comprise the administration of one or more additional cancer therapies, such as, but not limited to, chemotherapies, and/or another radiotherapy.

Pharmaceutical Composition

Another object of the invention relates to a pharmaceutical composition comprising a polypeptide or nucleic acid according to the invention and a pharmaceutically acceptable carrier.

Typically, polypeptide or nucleic acid according to the invention may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions

"Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

In the pharmaceutical compositions of the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, local or rectal administration, the active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms 45 comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intransal administration forms and rectal administration forms.

Preferably, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium 55 phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the 65 form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of

12

manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

Solutions comprising compounds of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The methionine gamma-lyase can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetables oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating
the active polypeptides in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization.
Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The preparation of more, or highly concentrated solutions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small tumor area.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutane-

ous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The methionine gamma-lyase may be formulated within a therapeutic mixture to comprise about 0.0001 to 1.0 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 milligrams, or about 1 to 10 milligrams or even about 10 to 100 milligrams per dose or so. Multiple doses can also be administered.

In addition to the compounds of the invention formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration; liposomal formulations; time release capsules; and any other form currently used.

Another object of the invention relates to a pharmaceutical ²⁰ composition according to the invention comprising one or more enzyme cofactors. For example, said enzyme cofactor is a pyridoxal phosphate.

Another object of the invention relates to a pharmaceutical composition according to the invention comprising one or ²⁵ more chemotherapeutic or radiotherapeutic agents.

The invention will be further illustrated by the following examples and figures. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES

FIG. 1: Dose-effect cytotoxicity curve of the recombinant MGL-BL929 in HT29 human colon carcinoma cell line ³⁵ exposed to the enzyme for 48 hr. and 72 hr.

FIG. 2: Dose-effect cytotoxicity curve of the recombinant MGL-BL929 in normal skin fibroblasts exposed 48 hr. and 72 hr. to the enzyme.

FIG. 3A-B: Concentration (expressed in percent of control) of L-methionine in the supernatant of HT29, SKOV3, Jurkat, and Reh human tumor cell cultures exposed to various concentrations of MGL-BL929.

FIG. **4**A-B: Concentration (expressed in percent of control) of L-homocysteine in the supernatant of HT29, SKOV3, 45 Jurkat, and Reh human tumor cell cultures exposed to various concentrations of MGL-BL929.

EXAMPLES

Materials and Methods

Methods:

Characterization of the Methionine Gamma-Lyase (MGL-BL929) from *Brevibacterium aurantiacum* (*B. aurantiacum*; 55 ATCC 9175; Formerly *B. linens*)

Various aspects of metabolism and gene expression were studied by transcriptome analysis of *Brevibacterium aurantiacum* (ATCC 9175) cultured in the absence and in the presence of various sulfur-containing amino acids. Studies led to 60 identification within the *B. aurantiacum* genome of a gene (BL929) encoding a putative MGL.

Production of the *Brevibacterium aurantiacum* Methionine Gamma-Lyase (MGL-BL929)

Bacterial Strain and Growth Production

The Escherichia coli BL21 (DE3) production strain (Agilent technologies, Santa Clara, Calif.) was grown in Erlenm-

14

eyer at 30° C. with 200-250 rpm shaking in reconstituted Luria Bertani (LB) broth: 1% tryptone, 5% yeast extract (Fluka, St Louis Mo.), 1% NaCl resuspended in pure water, supplemented with 50 μ g/mL kanamycin when necessary. Solid media were prepared by adding technical agar (Invitrogen, Paisley, UK) at a final concentration of 1.5% w/v.

Production and Purification of the *Brevibacterium auran*tiacum Methionine Gamma-Lyase MGL-BL929

The open reading frame (ORF) encoding MGL-BL929 from B. aurantiacum was optimized in order to adapt B. aurantiacum codon usage to E. coli expression system (SEQ ID NO: 3). The optimized gene was synthesized and sub cloned into an E. coli subcloning vector given pMA-BL929, according to GeneART standard procedures. The BL929 ORF was digested by restriction enzymes NdeI and XbaI from pMA-BL929 and ligated into E. coli pGTPc608a expression vectors (pET9d derivative carrying PTac promoter, Lac operator, a multiple cloning site, LacIq and T1T2 terminator) previously digested by the same enzymes. Ligation mixture was transformed into electro-competent cells of E. coli DH5α strains. The resulting plasmids pGTPc608-BL929 were verified by digestion and sequencing. Restriction enzymes, T4 DNA ligase and Antartic phosphatase (New England Biolabs, Ipswich, Mass.), high fidelity PhusionTM DNA polymerase (Finnzymes, Espoo, Finland) were used according to recommendations of the manufacturers. DNA purification kits were purchased from Macherey-Nagel (Düren, Germany). Sequencing was performed by Genome Express (Meylan, France).

The *E. coli* production strain BL21 (DE3) was transformed with the expression plasmid pGTPc608-BL929. A single colony carrying pGTPc608-BL929 was grown at 37° C. in a 70 L Applikon bioreactor containing 35 L of GYP medium (yeast extract 24 g/L, soy peptone 12 g/L, KH $_2$ PO $_4$ 4.8 g/L, K $_2$ HPO $_4$ 2.2 g/L and glycerol 5 g/L) supplemented with 0.02% pyridoxal 5'-phosphate. Expression was induced by 0.1 mM IPTG when the culture reached an OD $_{600nm}$ of 2. When initial glycerol was totally depleted, concentrated feeding-solution (30% w/v glycerol and 30% w/v yeast extract) was added by a remote controlled pump. The pump flow rate was regulated based on pH set point. Cells were harvested when the culture OD $_{600nm}$ reached 40-50.

Cells were suspended in lysis buffer (20 mM Tris, 1 mM PMSF, 1 mM EDTA, 1 mM DTT, 0.1 mM pyridoxal 5'-phosphate, pH 7.5) and lysed by high pressure homogenization. The cell extract was clarified by centrifugation for 30 min at 15,900 g at 4° C. then filtered through a 0.45 μm low level protein binding disposable filter (Sartorius, Gottingen, Germany). Solid ammonium sulphate (AS) was added to the cell 50 extract to reach a final concentration of 0.75 M and stirred at 4° C. for 16 hours. The sample was centrifuged at 15,900 g for 30 min at 4° C., then filtered with a 0.45 μm low level protein binding disposable filter to remove all precipitated proteins before being injected in a column of Phenyl Sepharose (High Sub) 6 Fast Flow resin (GE Healthcare). The column was equilibrated with 20 mM Tris, 0.75 M AS, 0.1 mM PLP, pH 7.5. Proteins were eluted by decreasing the AS concentration in several steps from 100% to 0%. The elution fractions containing MGL-BL929 were pooled, concentrated 5-fold using an ultrafiltration cassette with a cut-off of 10 kDa, then diafiltered versus 7 volumes of 20 mM Bis Tris, 0.1 mM PLP, pH 6.0 (Pall, Port Washington, N.Y.).

The Hydrophobic Interaction Chromatography (HIC) elution was passed through a column packed with DEAF Sepharose Fast Flow resin (GE Healthcare) pre equilibrated in 20 mM Bis Tris, 0.1 mM PLP, pH 6.0. Proteins were eluted by increasing NaCl concentration in several steps from 0 to 1

M. The elution fractions containing MGL-BL929 were collected, concentrated 3 fold using an ultra-filtration cassette (Pall, Port Washington, N.Y.) then diafiltered versus 7 volumes of 40 mM Na*PO4, 20 µM PLP, pH 7.5.

The DEAF elution was clarified by filtration through a 0.2 5 µm low level protein binding disposable filter (Corning, Union City, Calif.). Endotoxins were removed by filtration through a Mustang E capsule (Pall, Port Washington, N.Y.). The concentration of MGL-BL929 was achieved using a stirred cell containing a membrane with a cut-off of 10 kDa in order to reach a final concentration range between 20 and 30 mg/mL (Millipore, Billerica, Mass.). The purified protein was stored at -80° C. Enzyme concentration and purity were determined by capillary electrophoresis, SDS PAGE and Bradford assay. The oligomerization state of the purified enzyme was investigated by Size Exclusion Chromatography (SEC) using a Superdex 200 5/150 GL column (GE Healthcare). Endotoxin concentration of the final product was assayed by Lonza (Testing services).

Enzyme Assays

The ability of the purified enzyme to catalyze α,γ elimination reactions against amino acids and substituted amino acids was tested separately with L-methionine, L-homocysteine, L-cysteine, L-cystathionine, S-adenosyl L-methionine, S-adenosyl L-homocysteine, and D-methionine. Activity was measured by incubating the enzyme in 100 mM K*PO₄, 0.01 mM PLP, pH 7.5 with 25 mM of substrate in a final volume of 1 mL. The mixture was incubated at 25° C. for 10 min, and the reaction was stopped by addition of trichloroacetic acid to a final concentration of 5%. The α -ketobutyrate formed in the supernatant solution was measured with 3-methyl-2-benzothiazolone hydrazone using spectrophotometry at 320 nm (Soda et al., 1967).

Cytotoxicity Studies and Cell Lines

Seven human tumor cell lines were used to determine the cellular effects of MGL-BL929. These comprise five adherent solid tumor-derived cell lines (including the colorectal carcinoma cell line HT29, two stable variants of HT29 selected for their high levels of resistance to methotrexate (HT29 $_{MTX}$), and to fluorouracil (HT29 $_{FUra}$), the ovarian carcinoma cell line SKOV3, and the hepatoblastoma-derived cell line (HepG2), and 2 non-adherent tumor cell lines (including the T-cell lymphoma cell line Jurkat, and the acute lymphocytic leukemia cell line Reh). Similarly, human nontumor skin fibroblasts (CDGII) were exposed to MGL-BL929 at various concentrations in order to explore selectivity of MGL-BL929 towards cancer cells.

The cells lines were thawed from mycoplasma-free frozen stocks and were controlled for contamination. Cells were maintained in cell culture medium (RPMI 1640 or DMEM according to the growth requirements of each cell line), supplemented with 10% FBS and antibiotics (streptomycin, 50 μg/mL, and penicillin, 50 U/mL) at 37° in an atmosphere containing 5% CO₂. Cells were exposed in 12 well-cell plates

16

to the purified recombinant MGL-BL929 at a concentration range from 0.05 U/mL to 5 U/mL, and were harvested 24 hr., 48 hr., 72 hr., and 96 hr. from start of the exposure. Cell viability was measured with the Trypan blue dye exclusion test in Malassez chambers.

Measurement of L-Methionine and L-Homocysteine in Cell Culture

The concentrations of L-methionine and L-homocysteine were measured in the supernatant of HT29, SKOV3, Jurkat, and Reh cell cultures growing exponentially in cell culture medium supplemented with 10% FBS, exposed at t0 to various concentrations of MGL-BL929 for 24 hr., 48 hr., 72 hr., and 96 hr. HT29 was grown in DMEM, and the three other cell lines were in RPMI 1640 cell culture medium. Measurements were done by ion-exchange chromatography. (N.B., concentrations of L-homocysteine in RPMI 1640, and in DMEM cell culture media are 101 μ mol/L, and 201 μ mol/L, respectively).

Studies of Protein Stability by Western Blot

Fractions of the supernatants of HT29 cells in culture exposed to various concentrations of the recombinant MGL-BL929 were subjected to SDS-PAGE under denaturing conditions, and then revealed by Western Blot (WB) with a polyclonal rabbit anti MGL-BL929 serum that was generated against the purified enzyme

Western Blot analysis was also performed in human scrum incubated with the purified recombinant MGL-BL929 for 72 hr

Results:

Characterization of the Methionine Gamma-Lyase (MGL-BL929) from *Brevibacterium aurantiacum* (*B. aurantiacum*; ATCC 9175; Formerly *B. linens*)

Comparisons of *Brevibacterium aurantiacum* (ATCC 9175) gene expression profiles in the presence of L-cystine and L-cystine plus L-methionine showed up-regulation of two adjacent genes in the presence of methionine, including the gene BL929 encoding a putative MGL. Up-regulation of BL929 was accompanied by the production of large amounts of volatile sulfur compounds resulting from degradation of methionine (Forquin et al., 2010).

The sequence of the putative BL929 gene was identified within the genome of *B. linens* (*Brevibacterium linens* BL2 NZ_AAGP01000007, whole genome shotgun sequence). The genome comprises 115535 base pairs (bp). The putative MGL nucleotide sequence BL929 is at position 34415 and is composed of 1182 bp (34415 to 35596) (SEQ ID NO: 2). The reference of the sequence of the translated protein is ZP_05913004.1 (SEQ ID NO: 1).

Purification

Purification of MGL-BL929 was achieved to homogeneity from a 5 L scale cell pellet after four successive chromatography steps (Table 1). The purification schema allowed purifying 1.2 g of MGL-BL929 with a degree of protein purity of 94% containing very low levels of endotoxin, corresponding to a recovery rate of 23%.

TABLE 1

| Purification of MGL-BL929 from Brevibacterium aurantiacum (ATCC 9175). | | | | | | | | | | |
|--|---------------------------|----------------------------|---------------------------|------------|----------------------------|-------------------------|--|--|--|--|
| Purification step | Total conc. (mg/mL) | [MGL- BL929] (mg/mL) | MGL- BL929 % purity | Amount (g) | Recovery Process (%) | Recovery Step (%) | | | | |
| Cell extract | 5.7 | 1.4 | 24 | 5.1 | | | | | | |
| (NH ₄) ₂ SO ₄ Precipitation | 3.4 | 1.1 | 32 | 3.9 | 76 | 76 | | | | |
| Phenyl Sepharose HIC | 0.2 | 0.1 | 64 | 2.5 | 49 | 64 | | | | |
| Concentration Diafiltration FF | 4.0 | 1.4 | 70 | 2.1 | 41 | 85 | | | | |
| DEAE Sepharose AEC | 1.1 | 0.9 | 81 | 1.3 | 25 | 60 | | | | |
| | | | | | | | | | | |

TABLE 1-continued

| Purification of MGL-BL929 from Brevibacterium aurantiacum (ATCC 9175). | | | | | | | | | | |
|--|---------------------------|----------------------------|---------------------------|-------------------|----------------------------|-------------------------|--|--|--|--|
| Purification step | Total conc. (mg/mL) | [MGL- BL929] (mg/mL) | MGL- BL929 % purity | Amount (g) | Recovery Process (%) | Recovery Step (%) | | | | |
| Concentration Diafiltration UF Mustang E Concentration | 2.2 1.6 23.7 | 2.2 1.6 22.3 | 98 100 94 | 1.0 0.9 1.2 | 20 17 23 | 80 86 100 | | | | |

Enzyme Size

The molecular weight of the native enzyme was investigated by size exclusion chromatography (SEC). The hydrodynamic volume of the purified MGL-BL929 corresponds to a 171.5 kDa globular protein. Since the molecular weight of MGL-BL929 determined by capillary electrophoresis under denaturing conditions is 46.5 kDa, the results suggest that MGL-BL929 is a monodisperse homotetrameric species.

Substrate Specificity

Enzymatic activity of the purified MGL-BL929 on various substrates was determined by measurement of α -ketoacids produced (Soda et al., 1967). The purified enzyme catalyzed the α , γ -elimination of L-methionine and L-homocysteine (Table 2). The enzyme had no activity towards L-cysteine, L-cystathionine, S-adenosyl-L-homocysteine and D-methionine. Affinity and specific activity of the purified MGL-BL929 for L-homocysteine were 7.3-fold, and 4.8-fold greater than that measured for L-methionine, respectively.

TABLE 2

| | e specificity and enzymatic ac L-BL929 from <i>Brevibacteriu</i> | |
|-----------|---|--------------------|
| Substrate | Km (mM) | Vmax (umol · min-1 |

| Substrate | Km (mM) | Vmax (µmol·min ⁻¹ ·m ⁻¹) |
|---------------------------|----------------|---|
| L-Homocysteine | 0.94 ± 0.8 | 27.7 ± 5.7 |
| L-Methionine | 6.83 ± 2.4 | 5.77 ± 1.4 |
| L-Cysteine | 0 | 0 |
| L-Cystathionine | 0 | 0 |
| S-Adenosyl L-homocysteine | 0 | 0 |
| D-methionine | 0 | 0 |

One unit of enzyme is the amount that catalyzes the formation of 1 μmol of $\alpha\text{-ketobutyrate}$ per minute

The Purified MGL-BL929 Exerts Strong Cytotoxic Activity Against Human Tumor Cell Lines Growing in Culture.

MGL-BL929 Cytotoxicity appeared in most cell lines at levels greater than 0.5 U/mL of MGL-BL929 and was augmented with increasing concentrations of the enzyme. Table 3 summarizes the IC50 for each cell line at 72 hours of exposure, representing the concentration of the recombinant 50 MGL-BL929 (in Units/mL) achieving 50% growth inhibition in tumor and normal cells.

The IC50s differ in the various tumor cell lines; they are lowest in lymphoma- and leukemia-derived cells and the standard colon carcinoma HT29 cells. In the other tumor-derived cells, the IC50s are comprised between 1 and 1.5 U/mL. Interestingly, the IC50 for MGL-BL929 in HT29 $_{MTX}$ was low (0.3 U/mL) when cells were simultaneously exposed to the enzyme and to 1 μ M methotrexate (MTX) to which they are fully resistant, which suggests that MGL-BL929 may 60 overcome prior resistance to cytostatic agents. Results highly suggesting potentiation of 5-fluorouracil (FUra) by MGL-BL929 have been obtained in the human colon carcinoma cell line HT29. From these results, the inventors conclude that MGL-BL929 is able to potentiate the cytotoxicity of cytostatic agents and can overcome prior resistance to these compounds.

TABLE 3

Growth inhibition in human cell lines in vitro exposed to the recombinant MGL-BL929 from *Brevibacterium aurantiacum* (ATCC 9175).

| Type of Cell | and normal fibroblasts exposed for 72 hours to the enzyme (in Units/mL) ¹ |
|-------------------------|--|
| HT29 | 0.9 |
| HT29 _{MTX} | $1.5 (0.3)^3$ |
| HT29 _{5-FU} | 1.2 |
| SKOV3 | 1 |
| HepG2 | 1.2 |
| Jurkat | 0.6 |
| Reh | 0.9 |
| Normal Skin Fibroblasts | >4 |

 1 One unit of MGL-BL929 is the amount that catalyzes the formation of 1 μmol of α-keto-butyrate per minute 2 IC50s for each cell type are extrapolated from the concentration-response curve

³IC50 of MGL-BL929 in HT29_{MTX} simultaneously exposed to 1 μM methotrexate (MTX) to which cells are fully resistant.

Exposure to MGL-BL929 of human non tumor fibroblasts CDGII affect their growth only at concentration levels greatly higher than that required for cytotoxicity of cancer cells. The inventors did not observe any measurable decrease in number of cells exposed to MGL-BL929 at concentrations up to 4 U/mL. At 5 U/mL was observed a slight inhibition of growth at 72 hr. from start of exposure to the enzyme, as shown in FIG. 2.

MGL-BL929 Produces Long Duration Depletion of L-Methionine and L-Homocysteine in the Supernatant of Human Tumor Cell Cultures.

Exposure to MGL-BL929 decreased rapidly the levels of methionine in the supernatant of all four cell lines studied, and the effect was augmented with increasing concentrations of the enzyme. The degree of methionine depletion achieved with a given concentration of MGL-BL929 was maintained during all the duration of the experiment, as shown in FIGS. 3A-B and 4A-B.

Figures are similar with L-homocysteine, the endogenous precursor of methionine, which originates from cells during their growth. As for L-methionine, L-homocysteine concentration in the supernatant of cell cultures strongly decreases under exposure to MGL-BL929, and the effect was augmented with increasing concentrations of the enzyme.

The Recombinant MGL-BL929 is Stable Under Tumor Cell Culture Conditions for Long Periods of Time.

The stability of the recombinant MGL-BL929 was studied in the supernatant of HT29 cell culture. Control is the purified recombinant MGL-BL929 at 1 U/mL in fresh DMEM medium. The supernatant of HT29 cells exposed to MGL-BL929 at 1 U/mL at t0 were harvested at 24 hr., 48 hr., 72 hr., and 96 hr. from start of the experiment.

Western Blot analysis performed with a polyclonal rabbit anti purified MGL-BL929 serum could not show any detectable protein alteration during 4 days under cell culture conditions. This finding, together with the persistent L-methion-

ine depletion for the same duration after a single exposure to the enzyme, indicates that MGL-BL929 is stable for long periods of time under these conditions.

19

The Recombinant MGL-BL929 is Stable in Human Serum in vitro for Long Periods of Time.

The stability of the recombinant MGL-BL929 was also studied in human serum in vitro by incubation of the serum with various amounts of the enzyme. As for the supernatant of tumor cell cultures, Western Blot analysis did not reveal any protein changes during 72 hr. from start of the incubation with the enzyme.

The inventors produced and purified one batch of 1.2 g of recombinant MGL-BL929 with a protein purity of 94% and low endotoxin levels (31 EU/mL). MGL-BL929 produced possesses high levels of L-methionine γ -lyase, and L-ho-

mocysteinase activity. The enzyme (a) depletes methionine and homocysteine from tumor-cell culture media for long periods, (b) is stable under tumor cell culture conditions, and in human serum in vitro, (c) possesses high cytotoxic activity against various human tumor cells growing in culture, (d) does not affect growth of normal cells at concentrations that are highly cytotoxic for cancer cells, (d) potentiate (modulate) the cytotoxic activity of cytostatic agents, and (d) circumvent prior cell resistance to cytostatics. Thus, the use of MGL-BL929 which presents methionine gamma-lyase and homocysteinase activity is a selective original approach to decrease tumor cell growth, potentiate the cytotoxic activity of cytostatic agents and circumvent prior cell resistance to cytostatics in cancer treatment.

SEQ ID NO: 1

| MTSLHPETLM | VHGGMKGLTE | AGVHVPAIDL | STTNPVNDVA | TGGDSYEWLA | TGHTLKDGDS | SEQ ID NO: 1 60 |
|---------------|------------|---------------|------------|------------|---------------|--------------------|
| AVYQRLWQPG | VARFETALAG | LEHAEEAVAF | ATGMAAMTAA | LLAAVSAGTP | HIVAVRPLYG | 120 |
| GSDHLLETGL | LGTTVTWAKE | ADIASAIQDD | TGLVIVETPA | NPSLDLVDLD | SVVSAAGNVP | 180 |
| VLVDNTFCTP | VLQQPISHGA | ALVLHSATKY | LGGHGDAMGG | IIATNADWAM | RLRQVRAITG | 240 |
| ALLHPMGAYL | LHRGLRTLAV | RMRAAQTTAG | ELAERLDAHP | AISVVHYPGL | KGQDPRGLLG | 300 |
| RQMSGGGAMI | AMELAGGFDA | ARSFVEHCNL | VVHAVSLGGA | DTLIQHPASL | THRPVAATAK | 360 |
| PGDGLIRLSV | GLEHVDDLAD | DLIAALDASR | AAA | | | 393 |
| | | | | | | SEQ ID NO: 2 |
| atgacctcac | tgcacccaga | aacgctcatg | gtccacggcg | gaatgaaagg | cctcaccgag | 60 |
| gcaggagtcc | acgtaccggc | catcgacctc | tcgaccacca | acccagtcaa | cgatgtcgcc | 120 |
| accggcggtg | actcctacga | atggctcgcc | accggccata | cgctcaagga | cggcgactcg | 180 |
| gccgtctacc | agegeetetg | gcagcccggt | gtcgcacgct | tegagacege | gctggccggg | 240 |
| ctcgaacacg | ctgaggaagc | agtegeette | gccacgggca | tggccgcaat | gactgccgca | 300 |
| cttctcgcgg | ccgtcagcgc | aggaacaccc | cacatcgtcg | cagtgcgtcc | cctctatggc | 360 |
| ggaagcgacc | acctcctcga | aaccggactg | ctggggacaa | cagtcacatg | ggcaaaggaa | 420 |
| gccgacatcg | cctcggcgat | ccaagatgac | accggactcg | tcattgtcga | gaccccggca | 480 |
| aaccccagcc | tggaccttgt | tgatctcgac | agtgtcgtct | cageegeegg | caacgtgcct | 540 |
| gtgctggtgg | acaacacatt | ctgcacacct | gttctccagc | agcccatctc | ccacggagcg | 600 |
| gccctcgtac | tgcacagcgc | gacaaaatac | ctcggcggtc | atggcgatgc | catgggcggc | 660 |
| atcatcgcca | ccaacgccga | ctgggcgatg | cgcctgcgac | aggtccgagc | catcacagga | 720 |
| gccctgctcc | accccatggg | cgcgtatctc | cttcatcggg | gcttgcgcac | tctggccgtg | 780 |
| cgcatgcgcg | cggctcagac | caccgccggt | gagetegetg | agcgcctgga | cgcgcaccct | 840 |
| gccatctccg | tcgtccacta | cccgggactg | aaaggccagg | acccacgcgg | actgctcgga | 900 |
| cgccaaatgt | ccggtggtgg | tgcgatgatc | gcgatggagc | tcgccggtgg | attcgacgcc | 960 |
| gcccgcagct | tcgtcgaaca | ctgcaacctc | gtcgtccacg | cggtgtccct | gggcggcgct | 1020 |
| gacactctca | tccagcatcc | ggcgtcactg | actcacaggc | cagttgcggc | cacggcgaag | 1080 |
| cccggcgatg | gtctcatccg | actctctgtg | ggactcgaac | acgtcgatga | cctggcagac | 1140 |
| at an aat aaa | taastaaaas | an agat an ta | attastaata | anntannaaa | t at an again | SEQ ID NO: 3 |
| | | | gttcatggtg | | | 60 |
| | | | agcaccacca | | | 120 |
| accggtggag | attcctatga | gtggctggcc | accggtcata | cgctgaaaga | tggtgatagc | 180 |
| gcagtttatc | agcgtctgtg | gcagcctggg | gttgctcgtt | ttgaaaccgc | tctggcaggg | 240 |

20

-continued

| 300 | gactgcagct | tggcagctat | gcaacgggta | tgttgcattt | ctgaagaggc | ctggagcatg |
|------|------------|------------|------------|------------|------------|------------|
| 360 | tctgtacggt | ctgtaagacc | cacattgttg | tggaacaccc | cagtttcagc | ctactggctg |
| 420 | ggctaaagag | cggttacttg | ctgggaacta | gaccggactg | atctgctgga | ggaagcgacc |
| 480 | aaccccggct | ttatagttga | accggactgg | tcaagacgat | caagcgctat | gcagatattg |
| 540 | taatgtaccc | cggcagcagg | teggttgttt | cgacctggat | tggatctagt | aatccttcac |
| 600 | ccatggagct | agcctatttc | gtcctgcagc | ttgtactccc | ataatacttt | gtcctggtcg |
| 660 | aatgggtggt | acggtgacgc | ctgggtgggc | tactaagtat | tgcattctgc | gcactggtcc |
| 720 | aattaccgga | aggttagagc | cgtctgagac | ttgggctatg | ccaatgcaga | attattgcaa |
| 780 | tctggcagta | gtctccgtac | ctacatcggg | tgcttacctg | atcctatggg | gcactgctgc |
| 840 | tgctcatccc | aacgtctgga | gaactggctg | caccgcaggg | ctgctcagac | cgtatgcgtg |
| 900 | actgctggga | atccccgtgg | aagggtcagg | teegggaetg | ttgttcatta | gcaatttccg |
| 960 | ctttgatgca | tggcaggggg | gctatggaac | ggcgatgatt | ccgggggggg | cgtcaaatgt |
| 1020 | gggtggtgct | ctgtatccct | gttgttcatg | ttgtaatctg | ttgttgagca | gcacgtagtt |
| 1080 | cacggcgaaa | cegtegegge | actcataggc | ggcttcgctg | ttcagcaccc | gatactctga |
| 1140 | tctggctgat | atgttgacga | ggactggagc | actqtcqqta | ggctgattag | cctqqtqacq |

REFERENCES

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

- Abuchowski A, van Es T, Palczuk NC, Davis F F. Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol. J Biol Chem. 35 1977 Jun. 10: 252(11):3578-81.
- Amarita F, Yvon M, Nardi M, Chambellon E, Delettre J, Bonnarme P. Identification and Functional Analysis of the Gene Encoding Methionine-γ-Lyase in *Brevibacterium linens*. Applied and Environmental Microbiology, 2004, 40 70: 7348-7354.
- 3. Chapman A P. PEGylated antibodies and antibody fragments for improved therapy: a review. Adv Drug Deliv Rev. 2002 Jun. 17; 54(4):531-45.
- Cunningham-Rundles C, Zhuo Z, Griffith B, Keenan J. Biological activities of polyethylene-glycol immunoglobulin conjugates. Resistance to enzymatic degradation. J Immunol Methods. 1992 Aug. 10; 152(2):177-90.
- Forquin-Gomez M. P., Hebert A, Roux A, Aubert J., Proux C., Heilier J. F., Landaud S., Junot C., Bonnarme P., Martin-Verstraete I. Global regulation of the response to sulfur availability in the cheese-related bacterium *Brevibacte*rium aurantiacum. Appl. Environ. Microbiol. 2011, 77:1449-1459.

- 6. Monfardini C, Schiavon O, Caliceti P, Morpurgo M, Harris J M, Veronese F M. A branched monomethoxypoly(ethylene glycol) for protein modification. Bioconjug Chem. 1995 January-February; 6(1):62-9.
- 7. Machover D, Zittoun J, Broet P, Metzger G, Orrico M, Goldschmidt E, Schilf A, Tonetti C, Tan Y, Delmas-Marsalet B, Luccioni C, Falissard B, Hoffman R. Cytotoxic synergism of methioninase in combination with 5-fluorouracil and folinic acid. Biochemical Pharmacology 2001, 61: 867-876.
- Machover D, Zittoun J, Saffroy R, Broet P, Giraudier S, Magnaldo T, Goldschmidt E, Debuire B, Orrico M, Tan Y, Mishal Z, Chevallier O, Tonetti C, Jouault H, Ulusakarya A, Tanguy M-L, Metzger G, Hoffman R M. Treatment of cancer cells with methioninase produces DNA hypomethylation and increases DNA synthesis. Cancer Research 2002, 62: 4685-4689.
- Soda, K. A spectrophotometric micro-determination of keto acids with 3-methyl 2-benzothiazolone hydrazone. Agric Biol Chem, 1967, 31: 1054-1060.
- Gavrish E. Yu., V. I. Krauzova, N. V. Potekhina, S. G. Karasev, E. G. Plotnikova, O. V. Altyntseva, L. A. Korosteleva, L. I. Evtushenko. 2004. Three New Species of Brevibacteria, *Brevibacterium antiquum* sp. nov., *Brevibacterium aurantiacum* sp. nov., and *Brevibacterium permense* sp. nov. Microbiology 73(no2): 176-183.

SEQUENCE LISTING

```
<160> NUMBER OF SEQ ID NOS: 3

<210> SEQ ID NO 1

<211> LENGTH: 393

<212> TYPE: PRT

<213> ORGANISM: Brevibacterium linens
```

<400> SEQUENCE: 1

·-

Met Thr Ser Leu His Pro Glu Thr Leu Met Val His Gly Gly Met Lys

-continued

| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
|------------|----------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Gly | Leu | Thr | Glu 20 | Ala | Gly | Val | His | Val 25 | Pro | Ala | Ile | Asp | Leu 30 | Ser | Thr |
| Thr | Asn | Pro 35 | Val | Asn | Asp | Val | Ala 40 | Thr | Gly | Gly | Asp | Ser 45 | Tyr | Glu | Trp |
| Leu | Ala 50 | Thr | Gly | His | Thr | Leu 55 | Lys | Asp | Gly | Asp | Ser 60 | Ala | Val | Tyr | Gln |
| Arg 65 | Leu | Trp | Gln | Pro | Gly 70 | Val | Ala | Arg | Phe | Glu 75 | Thr | Ala | Leu | Ala | Gly 80 |
| Leu | Glu | His | Ala | Glu 85 | Glu | Ala | Val | Ala | Phe 90 | Ala | Thr | Gly | Met | Ala 95 | Ala |
| Met | Thr | Ala | Ala 100 | Leu | Leu | Ala | Ala | Val 105 | Ser | Ala | Gly | Thr | Pro 110 | His | Ile |
| Val | Ala | Val 115 | Arg | Pro | Leu | Tyr | Gly 120 | Gly | Ser | Asp | His | Leu 125 | Leu | Glu | Thr |
| Gly | Leu 130 | Leu | Gly | Thr | Thr | Val 135 | Thr | Trp | Ala | Lys | Glu 140 | Ala | Asp | Ile | Ala |
| Ser 145 | Ala | Ile | Gln | Asp | Asp 150 | Thr | Gly | Leu | Val | Ile 155 | Val | Glu | Thr | Pro | Ala 160 |
| Asn | Pro | Ser | Leu | Asp 165 | Leu | Val | Asp | Leu | Asp 170 | Ser | Val | Val | Ser | Ala 175 | Ala |
| Gly | Asn | Val | Pro 180 | Val | Leu | Val | Asp | Asn 185 | Thr | Phe | Cys | Thr | Pro 190 | Val | Leu |
| Gln | Gln | Pro 195 | Ile | Ser | His | Gly | Ala 200 | Ala | Leu | Val | Leu | His 205 | Ser | Ala | Thr |
| Lys | Tyr 210 | Leu | Gly | Gly | His | Gly 215 | Asp | Ala | Met | Gly | Gly 220 | Ile | Ile | Ala | Thr |
| Asn 225 | Ala | Asp | Trp | Ala | Met 230 | Arg | Leu | Arg | Gln | Val 235 | Arg | Ala | Ile | Thr | Gly 240 |
| Ala | Leu | Leu | His | Pro 245 | Met | Gly | Ala | Tyr | Leu 250 | Leu | His | Arg | Gly | Leu 255 | Arg |
| Thr | Leu | Ala | Val 260 | Arg | Met | Arg | Ala | Ala 265 | Gln | Thr | Thr | Ala | Gly 270 | Glu | Leu |
| Ala | Glu | Arg 275 | Leu | Asp | Ala | His | Pro 280 | Ala | Ile | Ser | Val | Val 285 | His | Tyr | Pro |
| Gly | Leu 290 | Lys | Gly | Gln | Asp | Pro 295 | Arg | Gly | Leu | Leu | Gly 300 | Arg | Gln | Met | Ser |
| Gly 305 | Gly | Gly | Ala | Met | Ile 310 | Ala | Met | Glu | Leu | Ala 315 | Gly | Gly | Phe | Asp | Ala 320 |
| Ala | Arg | Ser | Phe | Val 325 | Glu | His | CÀa | Asn | Leu 330 | Val | Val | His | Ala | Val 335 | Ser |
| Leu | Gly | Gly | Ala 340 | Asp | Thr | Leu | Ile | Gln 345 | His | Pro | Ala | Ser | Leu 350 | Thr | His |
| Arg | Pro | Val 355 | Ala | Ala | Thr | Ala | 160 160 | Pro | Gly | Asp | Gly | Leu 365 | Ile | Arg | Leu |
| Ser | Val 370 | Gly | Leu | Glu | His | Val 375 | Asp | Asp | Leu | Ala | Asp 380 | Asp | Leu | Ile | Ala |
| Ala 385 | Leu | Asp | Ala | Ser | Arg 390 | Ala | Ala | Ala | | | | | | | |
| | 0> SI 1> LI | | | | | | | | | | | | | | |

<211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens

-continued

| atgacetcae tgeacecaga aaegeteatg gteeaeggeg gaatgaaagg ceteaecgag geaggagtee acgtacegge categacete tegaceacea aceagteaa egatgtegee 120 aceggeggtg actectacga atggetegee aceggecata egeteaagga eggegaeteg 180 geogtetace agegeetetg geageceggt gtegeaeggt tegagaegge getggeeggg 240 ctegaacacg etgaggaage agtegeette gecaegggea tggeegeaat gactgeegga 360 cttetegggg eggteagege aggaacace cacategteg cagtgegtee cetetatgge 360 ggaagegace acetectega aaceggactg etggggaaaa cagtcacatg ggeaaaggaa 420 geogacateg eteggegat caaagatgae aceggacatg teattgtega gaceceggea 480 aaceceagee tggacettgt tgatetegae aggteegte cattgtega gaceceggea 480 aceceagee tggacettgt tgatetegae aggteegte cattgtega gaceceggea 660 geoctegtae tgeacageg gacaaaatae eteggeggte atggeegae eteggeegg 660 aceatecge eacecatggg eggatace etteategg gettgeegae eteggeegg 772 geoctgetee aceceatggg eggatacte etteateggg gettgegae eteggeegg 772 geoctgetee aceceatggg eggatacte etteateggg gettgegae eteggeegg 772 geoctgetee aceceatggg eggatacte etteateggg gettgegae eteggeegg 772 geocatetee eggegeaga eacegggt gageteggag acecaeggg acegeacet 772 geocatetee eggegeaga eacegggt gageteggag eggategga 262 geocaatg eggeteaga eaceggggt gageteggag acecaeggg acegeage 772 geocagate tegtecaata eeggaatg gageteggag eaceaeggg acegeage 772 geocagate tegtecaata eeggaatg gageteggag eaceaeggg 272 gacactetea tecagaaca etgaaacte gegategaag eegaceggg 272 gacactetea tecagaaca eaceaetgg gagetegag eaggaggaga 272 gacactetea tecagaaca eaceaetgg gaceaegag 273 gacactetea tecagaaca eeggeateg 273 gacactetea tecagaaca eaceaetgg 273 gacactetea tecagaaca 273 gacactetea gegategaa 273 gacactetea tecagaaca 273 gacactetea eaceaetgg 273 gacactetea tecagaaca 273 gacactetea 273 gacactet | geaggagtee aegtacegge categacete tegaceacea aeceagteaa egatgtegee 120 aceggeggtg aetectaega atggetegee aeeggecata egeteaaga eggegateeg 240 ctegaacaeg etgaggaage agtegeette gecaceggea tegacege getgeegge 240 ctetaegae cegteagga agaacaece cacategteg cagtgegee etetatgee 240 geagacaeg etgaggaage agtegeette gecaceggea tegacegge 240 cteteagae cecteeggea aceagateg etgaggaaca cagtecaetg geaacgagae 240 geogacateg ecteggegat ceaagatgae aceggacteg teattgtega gaceceggea 240 aceceagee tggacettg tgatetegae agtgeette caceggaeg cacegtgee 2540 gtgetggtgg acaacacat etgacacet gttetecage ageceatete ecaeggaege 2540 gecetgetee tegaceggg gegatate etteateggg gettgegae etgaggegge 2540 acetgetee aceceatggg eggstate etteateggg gettgegeae tetggeegge 2540 gecetgetee aceceatggg eggstatee etteateggg gettgegeae tetggeegge 2540 geceateteeg tegtecacta ecegggaetg aggsteggtg aggetegga aceggeagee 2540 gecegaaget tegtegaaca etgeaacete gegtgeagg eeggtgateet gggeggeet 2540 gecegaaget tegtegaaca etgeaacete gegtgeagae gegtgateet ggggggaget 2540 geceggaaget tegtegaaca etgeaacete gegtgeagae aggtgagga etggeggaga 2540 geceggaaget tegtegaaca etgeaacete gegtgeagae aggtgagga etggeagae 2540 geceggaaget geteateeg acetetetgg ggactegaa aggtegatga etggeggaga 2540 geacetetaa tecagaace eggagtegg gecegtgae aggtgagga etggeagae 2540 geacetetae tecagaace gegagtegg gecegtgae acetggagga 2540 geacetgagga gteteateeg acetetegga gtteateggg gaatgaagg tetgacega 2540 geacetgaggae tegacetegg acetetegga gtteatgagg gaatgaagg tetgacega 2540 secggagate tagtecegga acetgatg gtteatggg gaatgaagg tetgacega 2540 acegggagate atectatag gtggetgge aceggtate geggaagae atectgaagge 2540 aceggagate atectatag gtggetgge acegggategg tegacagga 2540 aceggattate agegetetgg gaacetggg gttgetegt tegaagae 2540 aceggaatatg eagacetggg aceggaagae aceatgggagg 2540 aceggaatattg caagaeggae aceaggaatgg 2540 aceg | <400> SEQUENCE: 2 | |
|--|--|--|--|
| accegogate actectacea atgoetege accegocata egeteaaga eggegacteg 240 ctogacacae eggegatetegge getegecegg 240 ctogacacae etgagaaga agtegetete gecaeggea tegecogaat gactegeega 300 cttetegegg cogteageg aggaacaece cacategteg eagtegeega 360 ggaaggacae acctectega aaccegoateg etgaggaaa eagtecatag ggaaaggaa 420 gecaeateg ceteggegat ceaagatgaa 420 gecaeateg etgagette tegacaegge tegacettg teatetgag gacacaeggee 480 aaccecagee tggacettg tgatetega agtgetet eagcegegg cacaegggee 540 gtgetggtgg acaacacat etgacacae ttgtetecaga agcecatete ecaeggageg 660 gecetegtae tgacaageg gacaaaatae eteggegge atgegate eatggegge 660 atcategeea ecaecaegge gacaaaatae eteggegge atgegateg eatggegge 720 gecetgetee accecatggg eggetatete etteateggg gettgegaa etteggegge 780 egacatetee accecatggg eggetatee etteateggg gettgegaa eteggeggeg 780 egacatetee accecatggg eggetatete etteateggg gettgegaa eteggeggtg 780 egacatetee degeceggt gagetegga aggeetegga eggetaetee 480 gecateteega eggeteatae eegggaatg aggetegga eteggeggeg 290 eggeaacet 840 gecateteega eggetgggggggggggggggggggggggggg | acceggeggtg actectacga atggetegee acceggecata egeteaagga egegaeteg 180 geegtetace agegeetetg geageeegg gtegeaegge tegagaeegg gtegeaegge tegagaeegg ettgagaaga 300 ettgagaaag agtegeete geaacgee tegagaeegg ettgagaegg 360 ettgagaaag agtegeete geaacgee acceetetaga eaceggaeegg etggegeaegg etgeaegge gggaaeggae eaceteetega aaceggaeteg etggggaeaa eagteacatg ggaaaaggaa 420 geegacateg etegacettg tegatetegae acceggaeteg teattgtega gaceeggae 480 aaceceaagee tggacettg tegatetegae agtgeetge teatggeggae acagtgeete 540 ggeetgatg etgacettg tegatetegae agtgeetget eaceggaegg eacegtgeet 660 geectegtae tgcacagee gacaaaatac etegaeggeg atgeetgae etgagaegg 720 geectegtae tgcacagge gacaaaaatac etegaeggeg aggetegae etegagaegg 720 geectegtae eacecatgg egetatete etteateggg gettgegae etegagegge 730 egeatgege gegetaaea eacegeeggt gageteggae gettgegeae etegagegg 730 geetgetee accecatgg egetatete etteateggg gettgegeae tetggeegg 730 egeatgege gegetaaea cacegeeggt gagetegga gegetegga egegeaecet 840 geeateteeg tegteeata eccegggaetg aaggeegg acceacagegg actgetegga 900 egecaaatgt eeggtgggg tgegatgate gegatggg acceacagegg actgetegga 900 egecaaatgt eeggtggg tgegatgate gegatggg tegeacega eggeagaeg 1020 gacaatetea tecagaaca etgcaaceat gtegtecaag eggtgeeet ggggggeet 1020 gacaatetea tecagaaca eggagaegg acceacagegg actgeegga 1180 eccegaegatg gteteateeg acceagaggg geogetgeet ga 1180 gacatetea tecagaaca eggagatgg geogetgeet ga 1182 e212 yttps: DNA 213 > DRAMINSH: Brevibacterium linens e400 > SEQUENCE: 3 atgacetege tgaatecega gaegetgatg gtteatggg gaatgaagg tetgacegaa 60 getggagga attectatga gtggetggee accggtaata getgaagag tetgacega 2120 accggagga attectatga gtggedgge accggtaatg tggaagae tetgacegae 2120 accggatgaa attectatga gtggedgge accggtaata gegaagae tetgaegge 2120 accggatgaa attectatga gtggaacace accggetgatg ttgaagae tetgaegge 2120 accggatgaaga attectatga gtggaacace accggtegatg ttgaagae tetgaegge 2120 accggatgaagae attegaegga gtggaacace accgaetgg ttgaagae tetgaegga 2120 ggaagaeggae attgaagae tegaagaegg 2120 ggaagaeggae attgaagaeggaeggaeggaeggaagaagaegga | atgaceteae tgcacecaga aacgeteatg gtecaeggeg gaatgaaagg eeteaeegag | 60 |
| geogtetace agegeetetg geageceggt gtegeaeget tegagacege getggeegga 300 cttetegeag cegtaageag agtacecec cacategteg cagtegeaga ggaaagaaga agtegette gecaegggaa tggeegaat gacetgeegaa 300 cttetegeag cegtaageag aggaacacec cacategteg cagtegete cetetatgge 360 ggaagegace aceteetega aaceggactg etggggaaa cagtecetg ggaaaggaa 420 geegacateg ceteggegat ceaagatgac aceggacteg teattgtega gaceceggea 480 aaceccagec tggacettg tgatetegac aggtegete cageggegg caacgtgeet 540 gtgetggtgg acaacacat etgeacacet gttetecage agcecatete ceacggageg 600 geectegtac tgeacageg gacaaaatac eteggeggte atggegatg catagagegg 660 atcategeca ceacgegg gacaaaatac eteggeggte atggegatg catagagggg 720 geectgetec acecaeggg ggetatete etteateggg gettgegac eteggeegg 780 egeatggeg cageaceteg aggeetegga aggeetegga 290 geetgege 290 geetgege 290 geetgegac acecaeggg 290 geetgege 290 geetgege 290 geetgegac 290 geetgegace 290 geetgegace 290 geetgegace 290 geetgegaga 290 geetgeaga 290 geetgegaga 290 geetgegaga 290 geetgeaga 290 geetgegaga 290 geetgeaga 290 geetgeaga 290 geetgegaga 290 geetgeaga 290 geetgeet 290 geetgeaga 290 geetgeaga 290 geetgeaga 290 geetgeggagggggggggggggggggggggggggggggg | cceptatace agegeteteg geageceggt glegeaeggt tegageage getggeeggg 240 ctegaacacg etgaggaag agtegeette gecaegggaa tgacegeat gactgeegea 360 cttetegegg cegteageg aggaacace cacategteg eagtgete cetetatgge 360 ggaagegace acetectega aaceggactg etgggggaca cagteactg gecaagggaa 420 geegacateg cetegggat ccaagatgac aceggactg teatigtega gaceceggea 480 aaceceagec tggacettg tgatetegac agtgetget cageegeeg caaeggged 540 gtgetggtgg acaacacatt etgcacacet gttetecaga ageceatete caceggageg 660 accetegtac tgcacageg egacaaatac eteggeggt atggetgatg categgegge 660 actactegea ccaaegceg acagaatac cteggeggt atggetgatg categgegge 772 geectgetea aceceatggg eggtatete etteateggg gettgegaac etteggeeggt 780 cgcatggeg eggeteagac caceggeggt gageteggac gategeage 772 geectgetea aceceatgg eggtatete etteateggg gettgegaac tetggeeggt 780 cgcatgege eggeteagac cacegeoggt gageteggt gageteggac etteggeeget 780 cgcaatgt eeggtggtg tgegatgate gegatggag eccaeggag acecaeggg 780 cgcacatgte eggtegaac etgeagactg aaaggeeggg acecaeggeg 780 cgcacatgt eeggtggtg tgegatgat gegatggag eeggtgeet 780 geceageat tegtegaac etgeagacet gegatggag eeggtgg 780 ccegeagat tegtegaac etgeagacet gegatggag eeggtgg 780 ccegeagat tegtegaac etgeagacet gegatggag eeggtgg 780 ccegeagat tegtecaeta eegggatgag gegeteet 780 geacatetea tecageatec gegegeactg 780 ccegeagat getecatecg acetetetgt ggactegaac acetegggag 780 ccegeagat getecatecg acetetetgt ggactegaac aceteggag 780 ccegeagat getecatecg acetetetgt ggactegaac aceteggag 780 ccegeagat getecatecg acetetetgtg gaateagag 780 ccegeagat getecatecg acetetetgtg gaateagag 780 ccegeagat getecatecg 780 ccegeagat 780 ccegeaga | gcaggagtee aegtacegge categacete tegaceacea aeceagteaa egatgtegee | 120 |
| ctogaacacg otgaggaage agtegoctte gecacgggea tggeegaat gactgeegaa 300 cttetegegg cegteagege aggaacacce cacategteg cagtegote ectetatgge 360 ggaagegace accteetega aaceggactg ctggggacaa cagtecattg ggaaaaggaa 420 gecgacateg ceteggegt caaaggatga cagtgegte catetgtega gacceeggaa 480 aaceccagce tggacettg tgatetegac aggtegote cagegeegg caaaggged 540 gtgetggtgg acaacacatt etgcacacet gttetecage agcecatete ccaeggageg 600 gecetegtac tgcacagge gacaaaatac eteggeggte atggegatg categgegge 660 atcategeca ccaacgeega ctgggegatg egectgegac aggteegatg categaggeg 660 atcategeca ccaacgeega ctgggegatg egectgegac aggteegatg categaggegge 660 atcategeca ccaacgeega ctgggegatg egectgegac aggteegage cateacagga 720 gecetgetec acceatggg egegtatete ctteateggg gettgegace tetggeeggg 780 egectgetec acceatggg egegtatete ctteateggg gettgegace tetggeeggt 780 egeatggege eggstecaga caccgeegg gageteegaa aggeetgga egegaceet 840 gecateteeg tegtecaata eccgggactg aggetegga acceacggga actgetegga 900 egecaaatgt ceggtggtg tgegatgate gegatggag tegeggggg attegacga 290 egecaaatgt ceggtggtg tgegatgate gegatggag tegeggggg attegagae 296 gecegeaagt tegtegaaaa etgeaacete gtegtecaca eggtgeegeg 1020 gacactetea tecaggate ggegtaactg acteacagge eagtgeggg 1020 gacactetea tecaggate ggegtaactg acteacagge caggtgggg 1080 eccggaggg geteteateeg actetetgtg ggactegaac acgtgagg actgggaaga 1180 eccggegatg gteteateeg actetetgtg ggactegaac acgtgatga cetggeagac 1140 gateteateg etgeatecega egegagtegg gecgetgeet ga 1182 eccggegagt tegatecega gacggatggg gecgetgeet ga 1182 eccggaggt tegatecega gacgetgatg gtteatggg gaatgaaggg tetgacegaa 60 getggagtte atgteegga aattgatega gacggagggg atteetatega gacggaggg gtteatggg gaatgaaggg tetgacaga 120 accggtgagg atteetatga gacgetggg gtteatggg gaatgaaga teggagagg 120 accgggaggaggaggagggagggggggggggggggggg | ctegaacacg ctgaggaage agtegette gecaegggea tggeegeaat gactgeegea 3600 cttetegegg cegteageg aggaacacee cacategteg cagtgegtee cetetatgge 3600 ggaagegace acetectega aaceggactg etggggaaa cagteacatg ggcaaaggaa 420 gecagaateg ctegaggat ccaagatgae aceggactg teattgtega gacceeggea 480 aaceccagee tggacettgt tgatetegae agtgetget cageegeegg caacgtgeet 540 gtgetggtgg acaacaatat etgcacacet gttetecage ageccatete ccaceggageg 600 gecetegtae tgcacageg gacaaataac eteggegge atggetgggge categagegge 660 atcategeae ccaacgcega ctgggegatg egectggae aggteegage cateacagga 720 gecetgetee aceccatggg egegtatete etteateggg gettgegae tetgggeegtg 780 cgcatggeeg eggetcagae caceggagtg aggeteggag acecceggg gactectega aggecetgga eggetegga 990 cgcatatete etteateggg gettgegaa eteggeggtg 990 cgcaaatgt eeggtgggg tgegatgate gegatggag acecaegggg atteggage 990 cgcaaatgt eeggtggtg tgegatgate gegatggag tegeegggg atteggage 1020 gaccatetea tecaggatgg tgegatgate gegatggage eagtgeegga 1020 gaccatetea tecagcatee ggegteactg actcacagge cagtgeegga 1080 eceggagatg deteateeg acetetetgtg ggactegaa aegtegagg eaggggggg 1140 aceteateg etgecetega eggagteggg gegetgeet ga 1180 aceteateg etgecetega eggagteggg gecgetgeet ga 1180 aceteateg etgecetega acetetegae eggaggggggggggggggggggggggggggg | accggcggtg actcctacga atggctcgcc accggccata cgctcaagga cggcgactcg | 180 |
| cttetegegg cegteagege aggaacacce cacategteg cagtgegtee cetetatgge 360 ggaagegace accteetega aaceggactg etggggacaa cagteacatg ggeaaaggaa 420 gecgacateg ceteggegat ceaagatgac aceggacteg teattgtega gaceceggea 480 aaceceagee tggacettgt tgatetegac agtgtegtet cageegeegg caacgtgeet 540 gtgetggtgg acaacacatt etgeacacet gttetecage agcecatete ceaeggageg 600 gecetegtac tgeacageg gacaaaatac eteggeggte atggegatg categageg 660 atcategeca ceaaegeega etgggegatg egeetgegac aggeetggge cateacagga 720 gecetgetee aceceatggg eggstatete etteateggg gettgegace eteggeeggt 780 cgcategegg eggeteagac cacegeeggt gagetegetg aggeetgga eggeacect 840 gecateteeg tegtecacta ecegggactg aacgeeggg accaeggacect 840 gecateteeg tegtecacta ecegggactg aaggeeggg accaeggga etgegacecet 840 gecateteeg tegtecacta ecegggactg aggeteggag accaegggg actgetegga 900 egcecaaatgt eeggtggtgg tgegatgate gegatggage tegeegggg attegacece 960 gecegcaaget tegtegaaca etgeaacete gtegtecace eggtgeeet gggeggeget 1020 gacactetea tecageatee ggegteactg acteacagge eagttgegge aceggegaag 1080 eeggegatg gteteateeg actetetgtg ggactegaac acgtgatga eetggeagac 1140 gateteateg etgeeetega egegagtegg geegetgeet ga 1182 <1210 SEQ ID NO 3 1211 LENGTH: 1182 1221 TYPE: DNA 1232 ORGANISM: Brevibacterium linens 1400 SEQUENCE: 3 atgacetege tgcatecega gacgetgatg gtteatggtg gaatgaaggg tetgacegaa 60 getggagtte atgtecegga aattgatetg agcaccacca atccegteaa tgatgtega 120 aceggtggag atteetatga gtggetggee aceggteata egetgaaaga tggtgatage 180 geagtttate agegtetgtg geagectggg ttteetegtt ttgaaacege tetggeagg 180 eetactggetg cagatteage tggaacacce cacattgttg ttgaaacege tetggaagg 240 etggaageag etgtateage tggaacacce cacattgttg etgtaaagac tetgtaaggg 180 geagattate cagagegga tetgeace cacattgttg etgtaaagac tetgtaaaga 180 geagattate cagagegga gaceggactg etgggaacac ecggtaata eggtaaagac tetgtaagge 180 etactggetg cagttteage tggaacacce cacattgttg etgtaaagac tetgtaagac 180 geagatattg caageggtat teaagacga aceggactg tetgaaceggt tetgaaagac 180 etactggetg cagactgga gaceggactg etgggaacta eggtaaagac tetgaaagac 180 etactggetg aac | cttctcoggg cogtcagoge aggaacacce cacatogteg cagtgogtec cetctatgge ggaagogace acctectoga aacoggactg etggggaca cagtcactg ggcaaaggaa 420 gccagactag etcagagat caaagatga accggactg teattgtoga gaccccggca 480 aacoccagoc tggaccttgt tgatctcgac agtgetget cagecgcogg caacgtgoct 540 gtgctggtgg acaacactt etgcacacct gttetcoage agccatete coacggagcg 600 gccctcgtac tgcacagog gacaaaatac etcggcggt atggctggtg catcgagcg 720 gccctcgtac tgcacagog gacaaaatac etcggcggt atggctgagt catcgagogg 720 gccctcgtac tgcacagog cgcgstatete etcatcggg gettgogac etctggocgtg 780 cgcatgogc cggctcagac caccgggtg gagctcggtg aggcctggac etcggcgtgg 900 gccatctc etcatcggg gcttgogac etcggcgtg 900 cgcaatagt cggtcagaa caccacggg accacct 840 gccatctcog tegtccaata eccgggatg aaaggccaga accacagogg actgctcgga 900 cgccaaatgt eggtggtgg tgcgatgate gcgatgaga etcgcggggg attcgagc 900 gcccaaatgt eggtggtgg tgcgatgate gcgatgaga etcgcggggg attcgago 900 cgccaaatgt eggtggggg tgcgatgate ggtgtccacg eggtgtcct ggggggggg 1000 gcccgaatgt tegtcgaaca etgcaacctc gtcgtccacg eggtgtcct gggggggggg 1000 gcccgaatgt tegtcgaaca etgcaacctc gtcgtccacg eggtgtcct gggggggggg 1000 gcccgaatgt tegtcgaaca etgcaacct gtcgtcacag eagtgggg 200 gcccgaatg gtctcatccg gggggagggggggggggg | gccgtctacc agcgcctctg gcagcccggt gtcgcacgct tcgagaccgc gctggccggg | 240 |
| ggaaggacc actectega aaccggactg ctggggacaa cagtcacatg ggcaaaggaa 420 gccgacatcg cttggcgat ccaagatgac accggactcg tcattgtcga gaccccggca 480 aaccccagcc tggaccttgt tgatctcgac agtgtcgtct cagccgcgg caacgtgct 540 gtgctggtgg acaacacatt ctgcacacct gttctccagc agccatctc ccacggagcg 600 gccctcgtac tgcacagcg gacaaaatac ctcggcggtc atggcgatg catcacagga 720 gccctcgtac tgcacaggcg cggatactc cttcatcggg gcttgcgac catcacagga 720 gccctgctcc accccatggg cggtatctc cttcatcggg gcttgcgac ctctggccgg 780 cgcatctcg tcgtccacac ccacggggt gaggtcggt gaggtcggt aggcctgga cgcgcacct 840 gccatctcg tcgtccact ccacgggactg aaaggccagg acccacggg actgctcgga 900 cgcacaatgt ccggtgggt tgcgatgat gcgatggac tcgccggga attgcgcg 900 cgccaaatgt ccggtgggt tgcgatgat gcgatggag tcgccgggg attgcgcg 900 cgccaaatgt ccggtgggg tgcgatgat gcgatgagac tcgccgggg attgcgcg 960 gcccgcaact tcgtcgaca ctgcacacc ggcgtccact accacggag acccacggg acccacgaga 1020 gacactctca tccagcatcc ggcgtcactg accacaggg cagttgcgg cacgggagac 1140 gacactcta tccagcatcc ggcgtcactg accacagag cagttgcggc cacgggagag 1140 gacactcatcg ctgccccga acctetctgtg ggactcgaac acgtcgatag cctggcagaa 1140 gatctcatcg ctgccccga actetctgtg ggactcgaac acgtcgatga cctggcagaa 1140 gatctcatcg ctgccccga gacgctggg gccgctgcct ga 1140 sex 1182 c212 stype: DNA c213 sex 1182 c212 | ggaagcgacc acctectega accegactg etggggacaa cagtecatg ggcaaaggaa 480 accegacteg ceteggegt caaagtgac accegacteg teattgtega gacceeggec 540 gtgctggtgg acaacacat etgcacacct gttetecage agcecatete ceaggageg 600 gccetegtac tgcacacct ctgcacacct gttetecage agcecatete ceaggageg 600 gccetegtac tgcacageg gacaaaatac etgggggte atgggatg categagegg 720 gccetegtec accecatggg eggetatete ettcateggg gettgegac tetggeegtg 780 cgcategge gggetagac caccaggag 720 gccetegetec accecatggg eggetatete ettcateggg gettgegac tetggeegtg 780 cgcateggeg eggetagac caccagggg gggetagag accacacgg acgecacct 840 gccateteeg tegtecacta eccgggactg agagteggag accacacggg actgetegga 900 cgcaaaatgt ceggtggtg tgcgatgate gegatggage tegecaggg actgetegga 900 cgcaaaatgt ceggtggtg tgcgatgate gegatggage tegecaggg actgetegga 900 cgcaaaatgt ceggtggg tgcgatgate gegatggage tegecaggg actgetegga 900 cgcaaaatgt ceggtgggg tgcgatgate gcgatggage tegecaggg actgetegga 900 cgcaaaatgt ceggtggg tgcgatgat gggatgace gggtgteet gggggggect 1020 gacactetea tecagacac etgcaacce gtegtecaga eagttgegge cacggeggag 1080 cccggcagg gteteateeg actectegg ggactegaa actgaagge categagaga 1140 gateteateg etgecetega eggegtagg gecgetgeet ga 1182 4210 SEQ ID NO 3 4211 LENNTH: 1182 4212 TYPE: DNA 4213 ORGANISM: Brevibacterium linens 4400 SEQUENCE: 3 atgaceteg tgcatecega gacgetgatg gttetatggtg gaatgaagg tetgacegaa 60 gctggagtte atgtecegga aattgatetg agcaccaca atccgtcaa tgatgeega 120 accggtggag attectatga gtggetggec accggtata eggtagagg 240 ctggagatta agcgtctgtg gcagettygg gttgctgtt ttgaaaccg tetggaggg 240 ctggaagattate agcgtctgtg gcagettygg gttgctgtt ttgaaaccg tetggaggg 240 ctggaagaggac atctgaggg gacggaagg taggaaggac tetggaggg ggaaggacgac atctgctgga gaccggaat teggagata eggtaatgg ggaagcagg taatgaag 420 ggaagaggaca atctgctgga gaccggaat teggagata egggaagg taatgaag 420 ggaagaggaca atctgctgga gaccggaat teggagat egggaagg taatgaag 420 gaagaaggac atctgagga gaccggaat teggagaag accaggat teggagaga ataccaggat 480 ggaagaggac atcaaaggat tegaagat etggagaga aatgaggag 420 gaagaaggac accaaggatgg gaccaggat etggagaga aattaccagg | ctcgaacacg ctgaggaagc agtcgccttc gccacgggca tggccgcaat gactgccgca | 300 |
| geogracing ectegogogat ceasgatgae aceggacteg teattgtega gaceceggea 480 aaceceagee tggacettgt tgatetegae agtgtegtet cageegeegg caacegtgeet 540 gtgetggtgg acaacacatt etgeacacet gttetecage ageceatete ecaceggageg 600 geoctegtae tgeacageg gacaaaatae eteggeggte atggegatge categgegge 660 atcategeea ceaaegeega etgggegatg egeetgegae aggteegage catecacagga 720 geoctgetee aceceatggg egegtatete etteateggg gettgegeae tetggeegtg 780 egeatgegeg eggeteagae cacegeeggt gagetegetg aggeetggae eggeaceet 840 geoateteeg tegtecacta ecegggactg aaaggeeagg acecacegga acegeaceet 840 geocateteeg tegtecacta ecegggactg aaaggeeagg ecgeggag attegagee 900 egecaaaatgt eeggtgggg tgegatgate gegatggage tegeegggg attegagee 960 geocgeaget tegtegaaca etgeaacete gtegtecacg eggtgteeet gggeggeget 1020 gacactetea tecageatee ggegteactg acecacagge cagtgeggeg 200 gecaggagggggggggggggggggggggggggggggggg | georgaaceg eteggegat caaagagaa aceggacteg teattgtega gacecegged 540 georgacege teggacettgt tgatetegaa agtgegtet cagecegegg caacgtgeet 540 gtgetggtgg acaacacatt etgacacact gttetecage agcecatete ceaeggageg 660 georgaacege tegacagegg gacaaaatac eteggeggte atggegatge catagagegg 660 atcategeca ecaacgeegg gacaaaatac eteggeggte atggegatge catagagggg 720 georgacea ecaacgeegg eggetatete etteateggg gettgegaac tetggeegtg 780 egcatgegg gegetaagac cacecgeggt gagetegga gegetagga eggegaceet 840 geoateteeg tegtecacta ecegggactg aaaggeegg aceceeggg actgetegga 900 egcaaaatgt eeggtggtgg tgegatgate gegatggage tegeegggg attegaceet 900 egcaaaatgt eeggtgggg tgegatgate gegatggage tegeegggg attegaceg 960 georgaaacte tegtegaaca etgeaacete gtegtecacg eggtgteet gggeggeget 1020 gacactetea teeggaac etgeaacete gtegtecacg eggtgteet gggeggeget 1020 gacactetea teeggaac etgeaacete gtegtecacg eggtgteet gggeggggg 1140 gateteateg etgeegaac acteetgtg ggactegaac acgtgagag etgeagaga 1140 gateteateg etgeectega eggegagegg geegetgeet ga 1182 *2210 > SEQ ID NO 3 | cttctcgcgg ccgtcagcgc aggaacaccc cacatcgtcg cagtgcgtcc cctctatggc | 360 |
| aaccccagcc tggaccttgt tggatctcgac agtgtcgtct cagccgccgg caacgtgcct 540 gtgctggtgg acaacacatt ctgcacacct gttctccagc agcccatctc ccacggagcg 600 gccctcgtac tgcacagcg gacaaaatac ctcggcggtc atggcgatgc catgggcggc 660 atcatcgcca ccaacgccga ctgggcgatg cgcctgcgac aggtccgagc catcacagga 720 gccctgctcc acccatggg cgcgtatctc cttcatcggg gcttgcgcac tctggccgtg 780 cgcatgcgcg cggctcagac caccgcggt gagctcgcg aggcctgga cgcgcaccct 840 gccatctccg tcgtccacta cccgggactg aaaggccagg acccacgcgg actgctcgga 900 cgccaaaatgt ccggtgggg tgcgatgatc gcgatgagac tcgccggtgg attcgagcac 900 cgccaaatgt ccggtgggg tgcgatgatc gcgatgagac cacgcggg attcgagcac 960 gcccgcaact tcgtcgaaca ctgcaacct gtgtccacg cggtgtccct gggcggcgct 1020 gacactctca tccagcatcc ggcgtcactg accacaggc cagtgcggc accggcgaag 1080 cccggcgatg gtctcatccg actctctgtg ggactcgaac acgtcgatga cctggcagac 1140 gatctcatcg ctgccctcga cgcgagtcgg gccgctgcct ga 1182 <210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens <400> SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaagg tctgaccgaa 60 gccggggggga attcctatga gtggctgcc accggtcaaa accgcgaaga tggtgatagc 120 accggtggga attcctatga gtggctggc accggtcata cgctgaaaga tggtgatagc 120 accggtggga attcctatga gtggctgcc accggtcata cgctgaaaga tggtgatagc 120 accggtggga attcctatga gtggctggc accggtcata cgctgaaaga tggtgatagc 120 accggtggga attcctatga gtggctggc accggtcata cgctgaaaga tggtgatagc 130 gcagtttatc agctctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcagg 240 ctggaagatt ctagagagcg tgttgcattt gcaacgggta tgcagcgtat gactgcagt 300 ctactggctg cagttcagc tggaaacacc cacattgttg ctgtaagacc tctgtacggt 360 ggaagacgacc acttgtcgg gaccggactg tctgggaacta cggttacttg ggcaaaaga 420 gcagaatattg caagcgctat tcaagacgat tcaaagacg tcaaagacg 420 gcagaatattg caagcgctat tcaagacgat tcaaagacg tcaaagacg 420 gcagaatattg | guectegate tegacettegt tegatetegae agtgetegtet cageogogg caaegtgeet 600 geoctegate tegacacacat ctegacacact guettecage agoccatete coaeggageg 600 geoctegate tegacageg gacaaaatac cteggeggte atgegatege categgegge 660 atcacaggac cacacagegg cacaggeggate geoctegate aggecage catacaagga 720 geoctegate acceateggg cegetatete cttcateggg gettegae tetggecagt 780 cgaatgegge gegetcagae cacegeoggt gagetegae aggecetgga eggeacect 840 geoatctceg tegtcoacta coeggggateg aaaggecagg acceaceggg actgetegga 900 cgacaatteeg tegtcoacta coegggateg aaaggecagg acceaceggg actgetegga 900 cgacaatteeg tegtcoacac ctegacacet gtegtcoaeg eggtgeet tegtcogga attegacge 960 geocegaget tegtcgaaca ctegacacet gtegtcoaeg eggtgeet ggacacete tegtcgaaca ctegacacet gtegtcoaeg eggtgeet gggaggeget 1020 gacactetea tecagcatee ggegtacate gactegagg cagtgegge acceggagag 1080 cceggegatg gtetcateeg actectgtg ggactegaac acgtegatga cetggcagac 1140 gatectaateg etgecetega eggstgagg geogetect ga 1182 cc212> TPPE DNA cc212> T | ggaagcgacc acctcctcga aaccggactg ctggggacaa cagtcacatg ggcaaaggaa | 420 |
| gtgctggtgg acaacacatt ctgcacacct gttctccagc agcccatctc ccacggagcg 600 gccctcgtac tgcacagcg gacaaaatac ctcggcggtc atggcgatg catggcggc 660 atcactgcca ccaacgccga ctgggcgatg cgcctgcgac aggtccgagc catcacagga 720 gccctgctcc accccatggg cgcgtatctc cttcatcggg gcttgcgcac tctggccgtg 780 cgcatgcgcg cgggtcagac caccgcggt gagctcgctg agcgcctgga cgcgcaccct 840 gccatctccg tcgtccacta cccgggactg aaaggccagg acccacgcgg actgctcgaa 900 cgccaaaatgt ccgggtggg tgcgatgatc gcgatggagc tcgccggtg attcgaccc 960 gcccgaagct tcgtcgaaca ctgcaacctc gtcgtccacc ggcgtcactg accacgcgg attcgacgc 960 gcccgcagct tcgtcgaaca ctgcaacctc gtcgtccacg cggtgtccct gggcggcgct 1020 gacactctca tccagcatcc ggcgtcactg accacagcg cagttgcggc cacgggagag 1080 cccggcgatg gtctcatccg acctctctgtg ggactcgaac acgtcgatga cctggcagac 1140 gatctcatcg ctgccctcga cgcgggtcgg gccgctgcct ga 1182 <210 > SEQ ID NO 3 | gtgctggtgg acaacacatt ctgcacacct gttctccagc agcccatctc ccacggageg 600 gccctcgtac tgcacageg gacaaaaatac ctcggcggtc atggcgatg catggggggc 660 atcatcgcca ccacagegg gacggatg cgcctgcac aggtccagc catcacagga 720 gccctgctcc accccatggg cgcgtatctc cttcatcggg gcttgcgcac tctggccgtg 780 cgcatgcgc gggctcagac caccgcggt gagctcgctg aggcctgga cgcgcacct 840 gccatctccg tcgtccacta cccgggactg aaaggccagg acccacggg actgctcgga 900 cgccaaatgt ccggtgggg tgcgatgatc gcgatggag tcgccgggg actgctcgga 900 cgccaaatgt ccggtgggg tgcgatgatc gcgatggagc tcgccgggg attgacgcc 960 gcccgcaact tcgtcgaaca ctgcaacct gtcgtccacg cggtgtccct gggcggcgct 1020 gacactctca tccagcatcc ggcgtcactg accacageg cagttgcggc cacggcgaag 1080 ccggggagg gtctcatccg actctctgtg ggactcgaac acgtcgaac acggcgaag 1140 gatctcatcg ctgccccga cgcgagtcgg gccgctgcct ga 1182 ccggggagg ctgccctcga cgcgagtcgggggggggg | gccgacatcg cctcggcgat ccaagatgac accggactcg tcattgtcga gaccccggca | 480 |
| gccctcgtac tgcacagcgc gacaaaatac ctcggcggtc atggcgatgc catgggcggc 660 atcatcgcca ccaacgccga ctgggcgatg cgcctgcgac aggtccgagc catcacagga 720 gccctgctcc accccatggg cgcgtatctc cttcatcggg gcttgcgcac tctggccgtg 780 cgcatgcgcg cggctcagac caccgccggt gagctcgctg agcgcctgga cgcgcaccct 840 gccatctccg tcgtccacta cccggggactg aaaggccagg acccacgcgg actgctcgga 900 cgccaaaatgt ccggtggtgg tgcgatgatc gcgatggagc tcgccggtgg attcgacgc 960 gcccgcagct tcgtcgaaca ctgcaacctc gtcgtccacg cggtgtccct gggcggcgct 1020 gacactctca tccagcatcc ggcgtcactg actcacaggc cactggagg 1140 gatctcatcg ctgccctcga cgcgatgtcg ggactcgaac acgtcgatga cctggcagac 1140 gatctcatcg ctgccctcga cgcgatgtcg ggccgctgcct ga 1182 <210 > SEQ ID NO 3 <211 > LENGTH: 1182 <212 > TYPE: DNA <213 > ORGANISM: Brevibacterium linens <400 > SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa 60 gctggagtt atgtcccga aattgatctg agcaccaca atccgtcaa tgatgtcgca 120 accggtggag attcctatga gtggctggc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 180 ctggagcatg ctgaagagg tgttgcattt gcaacggta tggcagctat gactgcagt 300 ctactggctg cagtttcagc tggaacacc cacattgttg ctgtaagacc tctgtacggt 360 gcaagatattg cagtttcagc tggaacacc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaaga 420 gcagatattg caagcgctat tcaagacgat ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat ctgggaacta cggtaattg gacccaggct 480 gcagatattg caagcgctat tcaagacgat ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat ctgggaacta ccggaactgg ttaagctgg ttaagctggt 480 | geoctegtac tgeacagege gacaaaatac cteggeggte atggegatge catgggggge 660 atcategeca ccaacgecga ctgggegatg egectgegac aggteegage cateacagga 720 geoctgetec accecatggg egegtatete ctteateggg gettgegeac tetggeegtg 780 egeatgegeg eggeteagac cacegeeggt gagetegetg agegeetgga egegeacect 840 geoatcteeg tegtecacta eccegggatg aaaggeeagg acceaeggg actgetegga 900 egecaaatgt eeggtgggtg tgegatgate gegatggage tegeeggg attegaegee 960 geoeggaget tegtegaaca etgeaacete gtegteeaeg eggtgteeet gggeggeget 1020 gacactetea tecageatee ggegteaetg actacacagge cacgtgegga 1080 ecceggegatg gteteateeg actetetgtg ggactegaac acgtegatga ectggeagac 1140 gateteateg etgeectega egegatgegg geoegetgeet ga 1182 <210 > SEO ID NO 3 <211 > LENGTH: 1182 <212 > TYPE: DNA <213 > ORGANISM: Brevibacterium linens <400 > SEQUENCE: 3 atgacetege tgeatecega gacgetgatg gtteatggg gaatgaaggg tetgaecgaa 60 getggagtte atgteecgga aattgatetg agcacacca atceegteaa tgatgtegea 120 accggtggag atteetatga gtggetggee accggteata egetgaaaga tggtgatage 180 geagtttate agegtetgtg geagectggg gttgetegtt ttgaaacege tetggeaggg 240 etggageatg etgaagagge tgttgeattt geaacgggta tggeagetat gactgeaget 300 etactggetg cagtteeage tggaacacce cacattgttg etgtaagace tetgtaeggt 360 geaagatattg caagegetat teaagacgat eeggaacac atceggeta aaccegget 360 geaagatattg caagegetat teaagacgat eeggaacac eeggtaattgeget 240 etggageate tegaagagge tgttgeattt geaacgggta tggeagetat gactgeaget 300 etactggetg cagttteage tggaacacce cacattgttg etgtaagace tetgtaeggt 360 ggaagegace atctgetgga gaccggactg etgggaacta eggttaettg ggetaaagag 420 geagatattg caagegetat teaagacgat tegggaacta eggttaettg ggetaaagag 420 geagatattg caagegetat teaagacgat teggtggt teggaagat teatgtee 540 geactggtee tggatetatg egacetggat eggtgggg aaggtaage aatgatget 660 geactggtee tgeattetge tactaagata etgggtggg aaggtagge aatgggtggt 660 attattgeaa ceaatgeaga ttgggetatg egtetgaga aggttagge aatgaegg attacteegga 660 attattgeaa ceaatgeaga ttgggetatg etgetgaga aggttagea aattacegga 720 geactgetge atcetatggg tgettaeteeg etacategg getetegtae tetggaaga aattacegga 660 | aaccccagcc tggaccttgt tgatctcgac agtgtcgtct cagccgccgg caacgtgcct | 540 |
| atcategeca ceaaegeega etgggegatg egeetgegae aggteegage cateacagga 720 geeetgetee acceeatggg egegtatete etteateggg gettgegeae tetggeeggg 780 egeatgegeg eggeteagae cacegeeggt gageteegtg agegeetgga egegeaecet 840 geeateteeg tegteeacta ecegggaetg aaaggeeagg acceaegegg actgetegga 900 egecaaatgt eeggtggtgg tgegatgate gegatggae tegeegggg actgetegga 960 geeeggaagt tegtgaaca etgeaaecte gtegteeaeg eggtgteeet gggeggeget 1020 gacactetea tecageatee ggegteeaetg acteacagge eagtgegge eaeggegaag 1080 eeegggatg gteteateeg actetetgtg ggaetegaae acgtegatga eetggegaag 1140 gateteateeg etgeeetega egegategg geegetgeet ga 1182 eeg12> Type: DNA eeg12> Type: DNA eeg12> Type: DNA eeg12> Type: DNA eeg22> Type: DNA eeg23> ORGANISM: Brevibacterium linens eeggtgagg atteetateeg actetateg ageaceacea atceegteaa tgatgtegea 120 accggtgaga atteetatga gtggetggee accggteata egetgaaga tgggagata 120 accggtgaga atteetatga gtggetggee accggteata egetgaaaga tggtgatage 180 geagtttate agegtetgtg geageetggg gttgetegtt ttgaaacege tetggeaggg 240 etggaageatg etgaagagge tgttgeattt geaaegggta tggeagetat gaetgeaget 300 etactggetg eagtteage tggaacacee eacattgttg etgtaagaee tetgtaeggt 360 ggaagegaee atetgetgga gaecggaetg etgggaacta eggtaettg ggetaaaga 420 geagaatattg eaagegetat teaagaegat teaagaegg tetgaagaeg 420 geagaatattg eaagegetat teaagaegat teaagaegg 420 geagaatattg eaagegetat teaagaegat etggaacaee accggetaat eggtaattg ggetaaagag 420 geagaatattg eaagegetat teaagaegat etggaacaee accggeta etggaaagae 420 geagaatattg eaagegetat teaagaegat etggaacaee accggeta etggaaaaaeee 420 geagaatattg eaagegetat teaagaegat etggaacaeee accggaattg etggaaaaaeee 420 geagaatattg eaagegetat teaagaegat 420 geagaatattg eaagegetat teaagaegat accggaattg accgaattg accggaattg accggaattg accgaattg | atcategeca ceaacgeega etgggegatg egectgegac aggteegage catcacagga 720 gecetgetee accecatggg egegtatete etteateggg gettgegeac tetggeegtg 780 egeatgeegg eggeteagac cacegeeggt gageteegtg agegetegga egegeaceet 840 gecateteeg tegtecata ecegggaetg aaaggeeagg acceaegegg actgetegga 900 egecaaatgt eeggtgggg tgegatgate gegatggage tegeeggtgg attegaegee 960 gecegeaget tegtegaaca etgeaacete gtegtecaeg eggtgteeet gggeggeget 1020 gacactetea tecageatee gegeteatg acteacagge eagttgegge caeggegaag 1080 eceggegatg gteteateeg acteetegtg ggaetegaac aegtegatga eetggeagaa 1140 gateteateeg etgeeetga gegegteet ga 1140 gateteateeg etgeeetga gegegteet ga 1140 saceteate etgeeetga eggagteegg geegetgeet ga 1140 saceteate etgeeetga eggagteegg geegetgeet ga 1140 saceteategge etgeeteega eggagteegg geegetgeet ga 1140 saceteategge etgeeteega eggagteegg geegetgeet ga 1180 seelijk line 1182 seelijk li | gtgctggtgg acaacacatt ctgcacacct gttctccagc agcccatctc ccacggagcg | 600 |
| geoctgetee accecatggg egegtatete etteateggg gettgegeae tetggeegtg 780 egeatgegge eggeteagae eaccgeeggt gagetegetg agegeetgga egegeacect 840 geoateteeg tegtecaeta eccgggaetg aaaggeeagg acceaeggg actgetegga 900 egecaaatgt eeggtggtgg tgegatgate gegatggage tegeeggtgg attegaegee 960 geoegeaget tegtegaaca etgeaacete gtegtecaeg eggtgteeet gggeggeget 1020 gacactetea tecageatee ggegteaetg acteaeagge eagttgegge eacggegaagg 1140 gateteateg etgeetega ectetetgtg ggaetegaae acgtegatga eetggeagae 1140 gateteateg etgeeetega egegagtegg geogetgeet ga 1182 <2210 > SEQ ID NO 3 <211 > LENGTH: 1182 <212 > TYPE: DNA <213 > ORGANISM: Brevibacterium linens <400 > SEQUENCE: 3 atgacetege tgeatecega gaegetgatg gtteatggtg gaatgaaggg tetgaeegaa 60 getggagtte atgteeegge aattgatetg ageaecaeca atecegteaa tgatgtegea 180 geagtttate agegtetgtg geageetggg gttgetegtt ttgaaacege tetggeaggg 240 etggagcatg etgaagagge tgttgeattt geaaegggta tggeagetat gaetgeaget 300 etaetggetg eagttteage tggaacaece eacattgttg etgtaagaec tetgtaeggt 360 ggaagegaec atetgetgga gaeeggaetg etgggaacta eggttaettg ggetaaaaga 420 geagatattg eaagegetat teaagaegat acggaetat eggtaettg ggetaaaaga 420 geagatattg eaagegetat teaagaegat etgggaacta eggtaettg ggetaaaaga 420 geagatattg eaagegetat teaagaegat acggaetag etggtaettg ggetaaaaga 420 geagatattg eaagegetat teaagaegat acggaetag etggtaettg ggeagaeta eggtaeatgg 420 | cgcatgcgc cggctcagac caccgcggt gagctcgct gagcgctgga ctctggccgtg 780 cgcatgcgcg cggctcagac caccgccggt gagctcgctg agcgcctgga cgcgcaccct 840 gccatctccg tcgtccacta cccgggactg aaaggccagg acccaccggg actgctcgga 900 cgccaaaatgt ccggtggtgg tgcgatgatc gcgatggagc tcgccggtgg attcgacgcc 960 gcccgcaagct tcgtcgaaca ctgcaacctc gtcgtccacg cggtgtccct gggcggcgct 1020 gacactctca tccagcatcc ggcgtcactg actcacaggc cagttgcggc cacggcgaag 1080 cccggcgatg gtctcatccg actctctgtg ggactcgaac acgtcgatga cctggcagac 1140 gatctcatcg ctgccctcga cgcgagtcgg gccgctgcct ga 1180 cccggcgatg gtctcatccg actctctgtg ggactcgaac acgtcgatga cctggcagac 1140 gatctcatcg ctgccctcga cgcgagtcgg gccgctgcct ga 1180 cccggcgatg gtctcatccg actctctgtg ggactcgaac acgtcgatga cctggcagac 1140 gatctcatcg ctgccctcga cgcgagtcgg gccgctgcct ga 1182 <210 > SEQ ID NO 3 <211 > LENGTH: 1182 <212 > TYPE: DNA <213 > ORGANISM: Brevibacterium linens <400 > SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa 60 gctggaggttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca 120 accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct 300 ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat tcggtagtt cggcagcag taatgtaccc 540 gcactggtcg ataatacttt ttgtactccc gtcctgaaga agctatttc ccatggagct 660 attattgcaa ccaatgcaga ttgggctatg cgtctgagac aggttagagc aatgggtggt 660 attattgcaa ccaatgcaga ttgggctatg cgtctactcg gtcctgagc aatggtggt 660 attattgcaa ccaatgcaga ttgggttacc ctacactggg gtctccgtac tctggcagta 720 gcactgctgc atcctatgg tgcttacctg ctacactggg gtctccgtac tctggcagta 720 | gccctcgtac tgcacagcgc gacaaaatac ctcggcggtc atggcgatgc catgggcggc | 660 |
| cgcatgcgcg cgctcagac caccgccggt gagctcgctg agcgcctgga cgcgcaccct 840 gccatctccg tcgtccacta cccgggactg aaaggccagg accaccgcgg actgctcga 900 cgccaaatgt ccggtggtgg tgcgatgatc gcgatggagc tcgccggtgg attcgacgc 960 gcccgcagct tcgtcgaaca ctgcaacctc gtcgtccacg cggtgtccct gggcggcgct 1020 gacactctca tccagcatcc ggcgtcactg actcacaggc cagttgcggc cacggcgaag 1080 cccggcgatg gtctcatccg actctctgtg ggactcgaac acgtcgatga cctggcagac 1140 gatctcatcg ctgccctcga cgcgagtcgg gccgctgcct ga 1182 cc10 SEQ ID NO 3 c211 LENGTH: 1182 c212 TYPE: DNA c213 ORGANISM: Brevibacterium linens c400 SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggg gaatgaaggg tctgaccgaa 60 gctggagttc atgtcccgg aattgatctg agcaccacca atcccgtcaa tgatgtcga 120 accggtggag attcctatga gtggctggc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcagg 240 ctggagcatg ctgaagagg tgttcatg gcaaccacca accggtcata gactgcagg 240 ctggagcatg ctgaagagg tgttcatg tgcaaccacca atctgctgaaga 240 ctactggctg cagtttcagc tggaaccacc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctga gaccggactg ctgggaacta cggttacttg ggctaaaga 420 gcagatattg caagcgctat tcaagacgat tcaagacgat tcaagacgat 420 gcagatattg caagcgctat tcaagacgat accggactg ttatagttga aaccccggct 480 gcagatattg caagcgctat tcaagacgat accggactat tcaagacgat 420 gcagatattg caagcgctat tcaagacgat accggactg ttatagttga aaccccggct 480 | cgcatgcgcg cggctcagac caccgccggt gagctcgctg agcgcctgga cgcgcaccct 840 gccatctccg tcgtccacta cccgggactg aaaggccagg acccaccggg actgctcgga 900 cgccaaatgt ccggtgggg tgcgatgatc gcgatggagc tcgccggtgg attcgacgcc 960 gcccgcaaatgt ccggtgggg tgcgatgatc gcgttccacg cggtgtccct gggcggcgct 1020 gacactctca tccagcatcc ggcgtcactg accacaggc cacggcgaag 1080 cccggcgatg gtctcatccg actctctgtg ggactcgaac acgtcgatga cctggcagac 1140 gatctcatcg ctgccctcga cgcgagtcgg gccgctgct ga 1182 <pre><210</pre> | atcatcgcca ccaacgccga ctgggcgatg cgcctgcgac aggtccgagc catcacagga | 720 |
| gccatctccg tcgtccacta cccgggactg aaaggccagg acccacgcgg actgctcgga 900 cgccaaatgt ccggtggtgg tgcgatgatc gcgatgagc tcgccggtgg attcgacgcc 960 gcccgcagct tcgtcgaaca ctgcaacctc gtcgtccacg cggtgtccct gggcggcgct 1020 gacactctca tccagcatcc ggcgtcactg actcacaggc cagttgcgc cacggcgaag 1080 cccggcgatg gtctcatccg actctctgtg ggactcgaac acgtcgatga cctggcagac 1140 gatctcatcg ctgccctcga cgcgagtcgg gccgctgct ga 1182 <210 > SEQ ID NO 3 <211 > LENGTH: 1182 <212 > TYPE: DNA <213 > ORGANISM: Brevibacterium linens <400 > SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa 60 gctggagttc atgtcccgca aattgatctg agcaccaca atcccgtcaa tgatgtcgca 120 accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagaggc tgttgcattt gcaacggta tggcagctat gactgcagt 300 ctactggctg cagtttcagc tggaacacc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgg gaccggactg ctgggaacta cggttacttg ggctaaaga 420 gcagatattg caagcgtat tcaagacgat accggactg ttatagttga aaccccggct 480 | gccatctccg tcgtccacta cccgggactg aaaggccagg acccacgcgg actgctcgga 900 cgccaaatgt ccggtggtgg tgcgatgatc gcgatggacc tcgccggtgg attcgacgcc 960 gcccgcagct tcgtcgaaca ctgcaacctc gtcgtccacg cggtgtccct gggcggcgct 1020 gacactctca tccagcatcc ggcgtcactg actcacaggc cactgtggcg cacgggaag 1080 cccggcgatg gtctcatccg actctctgtg ggactcgaac acgtcgatga cctggcagac 1140 gatctcatcg ctgccctcga cgcgagtcgg gccgctgcct ga 1182 <210 SEQ ID NO 3 <211 LENGTH: 1182 <212 TYPE: DNA <213 ORGANISM: Brevibacterium linens <400 SEQUENCE: 3 atgacetcgc tgcatccgga attgatctg agcacacca atcccgtcaa tgatgtcgca 120 accggtgaga attcctatga gtgctggcc accggtcata cgctgaaaga tggtgatagc 180 gcagttata agcgtctgtg gcagctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagagcg tgttgcattf gcaacgggta tggcagctat gactgcagc 300 ctactggctg cagttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgag gaccggactg ctgggaacta cggttaatgg gctaaagag 420 gcagaatattg caagcgctat tcaagacgat accggactgg ttatagttg accccggct 480 aatccttcac tggatctagt cgacctggat tcgggaacta cggttacttg ggcaacacc 540 gtcctggtcg ataatacttt ttgtaaccc gtcctgaaga agcctattc ccatggagct 660 gcactggtcg tactaatgctg tactaagtat ctgggtggc acggtgacga aatggtggt 660 attattgcaa ccaatgcaga ttgggctat cgtctgaaga aggttaagac aattaccgga 720 gcactgctgc atcctatggg tcgctaatg cgtctaatgg ctctgaagac aggttaagac aattaccgga 720 gcactgctgc atcctatggg tcgctaatcg ctctgaagac aggttaagac aattaccgga 720 gcactgctgc atcctatggg tcgctaatccg ctacaacggg tcacaacgga aggttaagac aattaccgga 720 gcactgctcc atcctatggg tcgctaatccg ctacaacgga aggttaagac aattaccgga 720 gcactgctcc atcctatggg tcgctaatccg ctacaacggg tcacaacgga aggttaagac aattaccgga 720 gcactgctcc atcctatggg tcgctaatccg ctacaacggg tcacaacgga aggttagagc aattaccgga 720 gcactgctcc atcctatggg tcgctaacccg ctacaacggg tcacaacgga aggttagac accaatgcaga 720 gcactgctcc atcctatggg tcgctaacccg ctacaacggg gtctccctacaacga 720 gcactgctcc atcctatggg tcgctaacccg ctacaacggg gtctcactgg gtctcacacacaacgcaga accaacacacacacacacacacac | gccctgctcc accccatggg cgcgtatetc cttcatcggg gcttgcgcac tctggccgtg | 780 |
| cgccaaatgt ccggtggtgg tgcgatgatc gcgatgagac tcgccggtgg attcgacgcc 960 gcccgcagct tcgtcgaaca ctgcaacctc gtcgtccacg cggtgtccct gggcggcgct 1020 gacactctca tccagcatcc ggcgtcactg actcacaggc cagttgcggc cacggcgaag 1080 cccggcgatg gtctcatccg actctctgtg ggactcgaac acgtcgatga cctggcagac 1140 gatctcatcg ctgccctcga cgcgagtcgg gccgctgcct ga 1182 <210 > SEQ ID NO 3 <211 > LENGTH: 1182 <212 > TYPE: DNA <213 > ORGANISM: Brevibacterium linens <400 > SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gtcatggtg gaatgaaggg tctgaccgaa 60 gctggagttc atgtcccga aattgatctg agcaccaca atcccgtcaa tgatgtcgca 120 accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagaggc tgttgcattt gcaacggta tggcagctat gactgcagct 300 ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgg gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactg ttatagttga aaccccggct 480 | cgccaaatgt ccggtggtgg tgcgatgatc gcgatggagc tcgccggtgg attcgacccc 960 gcccgcagct tcgtcgaaca ctgcaacctc gtcgtccacg cggtgtccct gggcggcgct 1020 gacactctca tccagcatcc ggcgtcactg actcacaggc cagttgcgc cacggcgaag 1080 cccggcgatg gtctcatccg actctctgtg ggactcgaac acgtcgatga cctggcagac 1140 gatctcatcg ctgccctcga cgcgagtcgg gccgctgcct ga 1182 c212> TYPE: DNA c213> ORGANISM: Brevibacterium linens c400> SEQUENCE: 3 atgacctcgc tgcatccacg actggtggg gttcatggg gatgaagag tcggaagac 120 accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc 120 accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagaggc tgttgcattt gcaacggata tggcagcta gactgcagct 300 ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactg ttatagttg accccggct 480 aatccttcac tggatctagt cgacctggat tcggtgttt tggcacctgg ttatagttg accccggct 480 aatccttcac tggatctagt cgacctggat tcggttgttt cggcagcagg taatgtaccc 540 gtcctggtcg ataatacttt ttgtactccc gtcctgcagc agcctatttc ccatggagct 660 attattgcaa ccaatgcaga ttgggctatg cgtctgagac acgttagac aatgggtggt 660 attattgcaa ccaatgcaga ttgggctatg cgtctaaccgg gtctccgtac tctggcagta 720 gcactgctc atcctatggg tgcttacctg ctacaatggg gtctccgtac tctggcagta 720 gcactgctc atcctatggg tgcttacctg ctacaatcggg gtctccgtac tctggcagta 720 gcactgctc atcctatggg tgcttacctg ctacaatggg gtctccgtac tctggcagta 720 gcactgctc atcctatggg tgcttacctg ctacaatcggg gtctccgtac tctggcagta 720 gcactgctc atcctatggg tgcttacctg ctacaatcggg gtctccgtac tctggcagta 720 gcactgctc atcctatggg tgcttacctg ctacaatcggg gtctccgtac tctggcagta 780 | cgcatgcgcg cggctcagac caccgccggt gagctcgctg agcgcctgga cgcgcaccct | 840 |
| geocgcaget tegtegaaca etgeaacete gtegtecaeg eggtgtecet gggeggeget 1020 gacactetea tecageatee ggegteactg acteacagge cagttgegge caeggegaag 1080 ceeggegatg gteteateeg actetetgtg ggactegaac acgtegatga eetggeagac 1140 gateteateg etgeeetega eggagtegg geogetgeet ga 1182 <210 > SEQ ID No 3 <211 > LENGTH: 1182 <212 > TYPE: DNA <213 > ORGANISM: Brevibacterium linens <400 > SEQUENCE: 3 atgacetege tgcatecega gaegetgatg gteatggtg gaatgaaggg tetgacegaa 60 getggagtte atgteeegge aattgatetg agcaceaea atceegteaa tgatgtegea 120 aceggtggag atteetatga gtggetggee aceggteata egetgaaaga tggtgatage 180 geagtttate agegtetgtg geageetggg gttgetegtt ttgaaacege tetggeaggg 240 ctggaageatg etgaagagge tgttgeattt geaacgggta tggeagetat gaetgeaget 300 ctactggetg eagtteage tggaacacee caeattgttg etgtaagae tetgtaeggt 360 ggaagegace atetgetgga gaeeggaetg etgggaacta eggttaettg ggetaaaga 420 geagatattg eaagegetat teaagaegat aceggaetg ttatagttga aacecegget 480 | gcccgcagct tcgtcgaaca ctgcaacctc gtcgtccacg cggtgtccct gggcggcgct 1020 gacactctca tccagcatcc ggcgtcactg actcacaggc cagttgcggc cacggcgaag 1080 cccggcgatg gtctcatccg actctctgtg ggactcgaac acgtcgatga cctggcagac 1140 gatctcatcg ctgccctcga cgcgagtcgg gccgctgcct ga 1182 <210 > SEQ ID NO 3 <211 > LENGTH: 1182 <212 > TYPE: DNA <213 > ORGANISM: Brevibacterium linens <400 > SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa 60 gctggagttc atgtcccga aattgatctg agcaccaca atcccgtcaa tgatgtcgca 120 accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaacacgc tctggcaggg 240 ctggaagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct 300 ctactggctg cagttcagc tggaacaccc cacattgttg ctgtaagacc tctgacaggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactg ttatagttga aaccccggct 480 aatccttcac tggatctagt cgacctggat tcggtgttt cggcagcagg taatgtaccc 540 gtcctggtcg ataatacttt ttgtactccc gtcctgcagc acggtgacgc aatgggtgt 660 attattgcaa ccaatgcaga ttgggctatg cgtctgagac aggttagagc aattaccgga 720 gcactgctgc atcctatggg tgcttacctg ctacatcggg gtctccgtac tctggcagta 780 gcactgctgc atcctatggg tgcttacctg ctacatcggg gtctccgtac tctggcagta 780 | gccatctccg tcgtccacta cccgggactg aaaggccagg acccacgcgg actgctcgga | 900 |
| gacactetea tecageatec ggegteactg acteacagge cagttgegge caeggegaag 1080 ceeggegatg gteteatecg actetetgtg ggactegaac acgtegatga cetggeagac 1140 gateteateg etgecetega egegagtegg geegetgeet ga 1182 <210 > SEQ ID NO 3 <211 > LENGTH: 1182 <212 > TYPE: DNA <213 > ORGANISM: Brevibacterium linens <400 > SEQUENCE: 3 atgacetege tgeatecega gaegetgatg gteatggtg gaatgaaggg tetgacegaa 60 getggagtte atgteeegge aattgatetg ageaceacea atecegteaa tgatgtegea 120 aceggtggag atteetatga gtggetggee aceggteata egetgaaaga tggtgatage 180 geagtttate agegtetgtg geageetggg gttgetegtt ttgaaacege tetggeaggg 240 ctggageatg etgaagagge tgttgeattt geaacggta tggeagetat gaetgeaget 300 ctaetggetg eagttteage tggaacacee caeattgttg etgtaagace tetgtaeggt 360 ggaagegace atetgetgga gaeeggactg etgggaacta eggttaettg ggetaaagag 420 geagatattg eaagegetat teaagacgat aceggactgg ttatagttga aacecegget 480 | gacactetea tecageatee ggegteactg acteacagge cagttgegge caeggegaag 1080 ceeggegatg gteteateeg actetetgtg ggactegaac acgtegatga cetggeagac 1140 gateteateg etgecetega egegagtegg geegetgeet ga 1182 <210> SEQ ID NO 3 | cgccaaatgt ccggtggtgg tgcgatgatc gcgatggagc tcgccggtgg attcgacgcc | 960 |
| cccggcgatg gtctcatccg actctctgtg ggactcgaac acgtcgatga cctggcagac 1140 gatctcatcg ctgccctcga cgcgagtcgg gccgctgcct ga 1182 <210 > SEQ ID NO 3 <211 > LENGTH: 1182 <212 > TYPE: DNA <213 > ORGANISM: Brevibacterium linens <400 > SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gtcatggtg gaatgaaggg tctgaccgaa 60 gctggagttc atgtcccga gacgctgatg gtcatggtg gaatgaaggg tctgaccgaa 120 accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct 300 ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaaga 420 gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct 480 | cccggcgatg gtctcatccg actctctgtg ggactcgaac acgtcgatga cctggcagac 1140 gatctcatcg ctgccctcga cgcgagtcgg gccgctgcct ga 1182 <210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens <400> SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa 60 gctggagttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca 120 accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagaaggc tgttgcattt gcaacgggta tggcagctat gactgcagct 300 ctactggctg cagttcagc tggaaccacc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaaga 420 gcagatattg caagcgctat tcaagacgat accggactg ttatagttga aaccccggct 480 aatccttcac tggatctagt cgacctggat tcggttgttt cggcagcagg taatgtaccc 540 gcactggtcg ataatacttt ttgtactccc gtcctgcagc agcctatttc ccatggagct 660 attattgcaa ccaatgcaga ttgggctatg cgtcgagca acggtgacg aattaccgga 720 gcactgctgc atcctatggg tgcttacctg ctacatcggg gtctccgtac tctggcagta 780 | gcccgcagct tcgtcgaaca ctgcaacctc gtcgtccacg cggtgtccct gggcggcgct | 1020 |
| gatetcateg etgecetega egegagtegg geogetgeet ga 1182 <210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens <400> SEQUENCE: 3 atgacetege tgeatecega gaegetgatg gtteatggtg gaatgaaggg tetgacegaa 60 getggagtte atgteeegge aattgatetg ageaceacea atecegteaa tgatgtegea 120 aceggtggag atteetatga gtggetggee aceggteata egetgaaaga tggtgatage 180 geagtttate agegtetgtg geageetggg gttgetegt ttgaacege tetggeaggg 240 etggageatg etgaagagge tgttgeattt geaaceggta tggeagetat gaetgeaget 300 etaetggetg eagttteage tggaacacee cacattgttg etgtaagace tetgtaeggt 360 ggaagegace atetgetgga gaeeggactg etgggaacta eggttaettg ggetaaagag 420 geagatattg eaagegetat teaagacgat aceggactgg ttatagttga aacecegget 480 | gatetcateg etgecetega egegagtegg geegetgeet ga 1182 <210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens <400> SEQUENCE: 3 atgacetege tgeatecega gaegetgatg gtteatggtg gaatgaaggg tetgacegaa 60 getggagtte atgtecegge aattgatetg ageaceacea atceegteaa tgatgtegea 120 aceggtggag atteetatga gtggetggee aceggteata egetgaaaga tggtgatage 180 geagtttate agegtetgtg geageetggg gttgetegtt ttgaaacege tetggeaggg 240 etggagcatg etgaagaagge tgttgeattt geaaceggta tggeagetat gaetgeaget 300 etactggetg eagttteage tggaacacee cacattgttg etgtaagace tetgtacggt 360 ggaagegace atetgetgga gaecegactg etgggaacta eggttacttg ggetaaaga 420 geagatattg eaagegetat teaagacgat aceggactg ttatagttga aacecegget 480 aateetteac tggatetagt egacetggat teggttgtt eggeageagg taatgtacee 540 gteetggteg ataatacttt ttgtacteee gteetgeage ageetattte ceatggaget 660 geactggtee tgeattetge tactaagtat etggtggge acggtgacge aatgggtggt 660 attattgeaa ceaatgeaga ttgggetatg egtetgagae aggttagage aattacegga 720 geactgetge ateetatggg tgettacetg etacateggg gteteegtae tetggeagta 780 | gacactetea tecageatee ggegteactg acteaeagge eagttgegge eaeggegaag | 1080 |
| <pre><210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens <400> SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa 60 gctggagttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca 120 accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct 300 ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct 480</pre> | <pre><210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens <400> SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa 60 gctggagttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca 120 accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagaggc tgttgcattt gcaacggta tggcagctat gactgcagct 300 ctactggctg cagttcagc tggaaccacc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct 480 aatccttcac tggatctagt cgacctggat tcggttgttt cggcagcagg taatgtaccc 540 gtcctggtcg ataatacttt ttgtactccc gtcctgcagc agcctatttc ccatggagct 660 gcactggtcc tgcattctgc tactaagtat ctgggtggc acggtgacgc aatgggtggt 660 attattgcaa ccaatgcaga ttgggctatg cgtctgagac aggttaagc aattaccgga 720 gcactgctc atcctatggg tgcttacctg ctacatcggg gtctccgtac tctggcagta</pre> | cccggcgatg gtctcatccg actctctgtg ggactcgaac acgtcgatga cctggcagac | 1140 |
| <pre><211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens <400> SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa 60 gctggagttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca 120 accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct 300 ctactggctg cagtttcagc tggaaccacc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct 480</pre> | <pre><211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens </pre> <pre><400> SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa 60 gctggagttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca 120 accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct 300 ctactggctg cagttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct 480 aatccttcac tggatctagt cgacctggat tcggttgttt cggcagcagg taatgtaccc 540 gtcctggtcg ataatacttt ttgtactccc gtcctgcagc agcctatttc ccatggagct 660 gcactggtcc tgcattctgc tactaagtat ctgggtggc acggtgacgc aatggtggt 660 attattgcaa ccaatgcaga ttgggctatg cgtctgagac aggttagagc aattaccgga 720 gcactgctgc atcctatggg tgcttacctg ctacatcggg gtctccgtac tctggcagta</pre> | | |
| atgacetege tgeatecega gaegetgatg gtteatggtg gaatgaaggg tetgacegaa 60 getggagtte atgteeege aattgatetg ageaceacea atecegteaa tgatgtegea 120 aceggtggag atteetatga gtggetggee aceggteata egetgaaaga tggtgatage 180 geagettate agegtetgtg geageetggg gttgetegtt ttgaaacege tetggeaggg 240 etggageatg etgaagagge tgttgeattt geaaceggta tggeagetat gaetgeaget 300 etactggetg eagttteage tggaacacee eacattgttg etgtaagace tetgtaeggt 360 ggaagegace atetgetgga gaeeggaetg etgggaacta eggttaettg ggetaaagag 420 geagatattg eaagegetat teaagaegat aceggaetg ttatagttga aaceeegget 480 | atgacetege tgcatecega gaegetgatg gtteatggtg gaatgaaggg tetgacegaa 60 getggagtte atgteeegge aattgatetg ageaceaeea atecegteaa tgatgtegea 120 aceggtggag atteetatga gtggetggee aceggteata egetgaaaga tggtgatage 180 geagettate agegtetgtg geageetggg gttgetegtt ttgaaacege tetggeaggg 240 etgagageatg etgaagagge tgttgeattt geaaceggta tggeagetat gaetgeaget 300 etactggetg eagtteage tggaaceaee eacattgttg etgtaagaee tetgtaeggt 360 ggaagegace atetgetgga gaeeggactg etgggaacta eggttaettg ggetaaagag 420 geagatattg eaagegetat teaagaegat aceggaetg ttatagttga aacecegget 480 aateetteae tggatetagt egaeetggat teggttgtt eggeageagg taatgtaeee 540 gteetggteg ataataettt ttgtaeteee gteetgeage ageetattte eeatggaget 600 geaetggtee tgeattetge tactaagtat etggtggge aeggtgaege aatgggtggt 660 attattgeaa ecaatgeaga ttgggetatg egtetgagae aggttagage aattaeegga 720 geaetgetge ateetatggg tgettaeetg etacateggg gteteegtae tetggeagta 780 | gateteateg etgeeetega egegagtegg geegetgeet ga | 1182 |
| gctggagttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca 120 accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct 300 ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct 480 | getggagtte atgteeegge aattgatetg ageaceacea atecegteaa tgatgtegea 120 aceggtggag atteetatga gtggetggee aceggteata egetgaaaga tggtgatage 180 geagtttate agegtetgtg geageetggg gttgetegtt ttgaaacege tetggeaggg 240 etgggageatg etgaagagge tgttgeattt geaaceggta tggeagetat gaetgeaget 300 etactggetg eagtteeage tggaacacee eacattgttg etgtaagace tetgtaeggt 360 ggaagegace atetgetgga gaeeggactg etgggaacta eggttaettg ggetaaagag 420 geagatattg eaagegetat teaagaegat aceggaetgg ttatagttga aacecegget 480 aateetteae tggatetagt egacetggat teggttgtt eggeageagg taatgtaece 540 gteetggteg ataataettt ttgtaeteee gteetgeage ageetattte eeatggaget 660 geaetggtee tgeattetge taetaagtat etgggtgge aeggtgaege aatgggtggt 660 attattgeaa eeaatgeaga ttgggetatg egtetgagae aggttagage aattaeegga 720 geaetgetge ateetatggg tgettaeetg etacateggg gteteegtae tetggeagta 780 | <210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA | 1182 |
| accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct 300 ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct 480 | accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc 180 gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct 300 ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct 480 aatccttcac tggatctagt cgacctggat tcggttgtt cggcagcagg taatgtaccc 540 gtcctggtcg ataatactt ttgtactccc gtcctgcagc agcctatttc ccatggagct 600 gcactggtcc tgcattctgc tactaagtat ctgggtggc acggtgacgc aatgggtggt 660 attattgcaa ccaatgcaga ttgggctatg cgtctgagac aggttagagc aattaccgga 720 gcactgctgc atcctatggg tgcttacctg ctacatcggg gtctccgtac tctggcagta 780 | <210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens | 1182 |
| gcagtttate agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct 300 ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct 480 | gcagtttate agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg 240 ctggagcatg ctgaagagge tgttgcattt gcaacgggta tggcagctat gactgcagct 300 ctactggctg cagtttcage tggaacacce cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct 480 aatccttcac tggatctagt cgacctggat tcggttgttt cggcagcagg taatgtacce 540 gtcctggtcg ataatacttt ttgtactcce gtcctgcage agcctatttc ccatggagct 600 gcactggtcc tgcattctge tactaagtat ctgggtggc acggtgacgc aatgggtggt 660 attattgcaa ccaatgcaga ttgggctatg cgtctgagac aggttagagc aattaccgga 720 gcactgctgc atcctatggg tgcttacctg ctacatcggg gtctccgtac tctggcagta 780 | <210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens <400> SEQUENCE: 3 | |
| ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct 300 ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct 480 | ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct 300 ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct 480 aatcettcac tggatctagt cgacctggat tcggttgttt cggcagcagg taatgtaccc 540 gtcctggtcg ataatacttt ttgtactccc gtcctgcagc agcctatttc ccatggagct 600 gcactggtcc tgcattctgc tactaagtat ctgggtggc acggtgacgc aatgggtggt 660 attattgcaa ccaatgcaga ttgggctatg cgtctgagac aggttagagc aattaccgga 720 gcactgctgc atcctatggg tgcttacctg ctacatcggg gtctccgtac tctggcagta 780 | <210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens <400> SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa | 60 |
| ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt 360 ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct 480 | ctactggetg cagtttcage tggaacacce cacattgttg ctgtaagace tetgtacggt 360 ggaagegace atetgetgga gaceggactg etgggaacta eggttacttg ggetaaagag 420 gcagatattg caagegetat teaagacgat aceggactgg ttatagttga aacecegget 480 aateetteae tggatetagt egacetggat teggttgttt eggeageagg taatgtacee 540 gteetggteg ataatacttt ttgtaeteee gteetgeage ageetattte eeatggaget 600 gcaetggtee tgeattetge tactaagtat etgggtgge aeggtgaege aatgggtggt 660 attattgeaa ecaatgeaga ttgggetatg egtetgagae aggttagage aattacegga 720 geaetgetge ateetatggg tgettaeetg etactategg gteteegtae tetggeagta 780 | <pre><210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens <400> SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa gctggagttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca</pre> | 60 120 |
| ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct 480 | ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag 420 gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct 480 aatccttcac tggatctagt cgacctggat tcggttgttt cggcagcagg taatgtaccc 540 gtcctggtcg ataatacttt ttgtactccc gtcctgcagc agcctatttc ccatggagct 600 gcactggtcc tgcattctgc tactaagtat ctgggtggc acggtgacgc aatgggtggt 660 attattgcaa ccaatgcaga ttgggctatg cgtctgagac aggttagagc aattaccgga 720 gcactgctgc atcctatggg tgcttacctg ctacatcggg gtctccgtac tctggcagta 780 | <pre><210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens <400> SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa gctggagttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc</pre> | 60 120 180 |
| geagatattg caagegetat teaagaegat aceggaetgg ttatagttga aacecegget 480 | gcagatattg caagegctat tcaagacgat accggactgg ttatagttga aaccccggct 480 aatcettcac tggatctagt cgacetggat teggttgttt eggeageagg taatgtacee 540 gteetggteg ataatacttt ttgtactcee gteetgeage ageetattte eeatggaget 600 gcactggtee tgcattetge tactaagtat etgggtgge aeggtgacge aatgggtggt 660 attattgeaa ceaatgeaga ttgggetatg egtetgagae aggttagage aattacegga 720 geactgetge atcetatggg tgettacetg etacateggg gteteegtae tetggeagta 780 | <pre><210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens <400> SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa gctggagttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg</pre> | 60 120 180 240 |
| | aatcetteae tggatetagt egacetggat teggttgttt eggeageagg taatgtacee 540 gteetggteg ataatacttt ttgtacteee gteetgeage ageetattte eeatggaget 600 geaetggtee tgeattetge tactaagtat etgggtgge aeggtgaege aatgggtggt 660 attattgeaa eeaatgeaga ttgggetatg egtetgagae aggttagage aattacegga 720 geaetgetge atcetatggg tgettaeetg etacateggg gteteegtae tetggeagta 780 | <pre><210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens <400> SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa gctggagttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg ctggagcatg ctgaagaggc tgttgcattt gcaacggta tggcagctat gactgcagct</pre> | 60 120 180 240 300 |
| aateetteae tggatetagt egacetggat teggttgttt eggeageagg taatgtacee 540 | gtcctggtcg ataatacttt ttgtactccc gtcctgcagc agcctatttc ccatggagct 600 gcactggtcc tgcattctgc tactaagtat ctgggtgggc acggtgacgc aatgggtggt 660 attattgcaa ccaatgcaga ttgggctatg cgtctgagac aggttagagc aattaccgga 720 gcactgctgc atcctatggg tgcttacctg ctacatcggg gtctccgtac tctggcagta 780 | <pre><210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens <400> SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa gctggagttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt</pre> | 60 120 180 240 300 360 |
| | gcactggtcc tgcattctgc tactaagtat ctgggtgggc acggtgacgc aatgggtggt 660 attattgcaa ccaatgcaga ttgggctatg cgtctgagac aggttagagc aattaccgga 720 gcactgctgc atcctatggg tgcttacctg ctacatcggg gtctccgtac tctggcagta 780 | <pre><210 > SEQ ID NO 3 <211 > LENGTH: 1182 <212 > TYPE: DNA <213 > ORGANISM: Brevibacterium linens <400 > SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa gctggagttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag</pre> | 60 120 180 240 300 360 420 |
| gteetggteg ataatacttt ttgtacteec gteetgeage ageetattte ceatggaget 600 | attattgcaa ccaatgcaga ttgggctatg cgtctgagac aggttagagc aattaccgga 720 gcactgctgc atcctatggg tgcttacctg ctacatcggg gtctccgtac tctggcagta 780 | <pre><210> SEQ ID NO 3 <211> LENGTH: 1182 <212> TYPE: DNA <213> ORGANISM: Brevibacterium linens <400> SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa gctggagttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaaccgc tctggcaggg ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag gcagatattg caagcgctat tcaagacgat accggactgg ttatagttga aaccccggct</pre> | 60 120 180 240 300 360 420 |
| gcactggtcc tgcattctgc tactaagtat ctgggtgggc acggtgacgc aatgggtggt 660 | gcactgctgc atcetatggg tgcttacctg ctacatcggg gtctccgtac tctggcagta 780 | <pre><210 > SEQ ID NO 3 <211 > LENGTH: 1182 <212 > TYPE: DNA <213 > ORGANISM: Brevibacterium linens <400 > SEQUENCE: 3 atgacetege tgcatecega gaegetgatg gttcatggtg gaatgaaggg tetgacegaa getggagtte atgteeegge aattgatetg ageaceacea atecegteaa tgatgtegea aceggtggag atteetatga gtggetggee aceggteata egetgaaaga tggtgatage geagtttate agegtetgtg geageetggg gttgetegtt ttgaaaeege tetggeaggg etggageatg etgaagagge tgttgeattt geaaegggta tggeagetat gaetgeaget etactggetg eagtteage tggaaeaeee eacattgttg etgtaagaee tetgtaeggt ggaagegace atetgetgga gaeeggaetg etgggaaeta eggttaettg ggetaaagag geagatattg eaagegetat teaagaegat aceggaetg ttatagttga aaeeeegget aateetteae tggatetagt egaeetggat teggttgttt eggeageagg taatgtaeee</pre> | 60 120 180 240 300 360 420 480 |
| attattgcaa ccaatgcaga ttgggctatg cgtctgagac aggttagagc aattaccgga 720 | | <pre><210 > SEQ ID NO 3 <211 > LENGTH: 1182 <212 > TYPE: DNA <213 > ORGANISM: Brevibacterium linens <400 > SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa gctggagttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaccgc tctggcaggg ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct ctactggctg cagtttcagc tggaaccacc cacattgttg ctgtaagacc tctgtacggt ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag gcagatattg caagcgctat tcaagacgat accggactg ttatagttga aaccccggct aatccttcac tggatctagt cgacctggat tcggttgttt cggcagcagg taatgtaccc gtcctggtcg ataatacttt ttgtactccc gtcctgcagc agcctatttc ccatggagct</pre> | 60 120 180 240 300 360 420 480 540 |
| geactgetge atectatggg tgettacetg ctacateggg gteteegtac tetggeagta 780 | | <pre><210 > SEQ ID NO 3 <211 > LENGTH: 1182 <212 > TYPE: DNA <213 > ORGANISM: Brevibacterium linens <400 > SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa gctggagttc atgtcccga aattgatctg agcaccacca atcccgtcaa tgatgtcgca accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaaccgc tctggcaggg ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct ctactggctg cagtttcagc tggaacaccc cacattgttg ctgtaagacc tctgtacggt ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag gcagatattg caagcgctat tcaagacgat accggactg ttatagttga aaccccggct aatccttcac tggatctagt cgacctggat tcggttgttt cggcagcagg taatgtaccc gtcctggtcg ataatacttt ttgtactccc gtcctgcagc accgtgaccg aatgggtggt gcactggtcc tgcattctgc tactaagtat ctgggtgggc acggtgaccg aatgggtggt</pre> | 60 120 180 240 300 360 420 480 540 600 |
| agtataggta atagtagga gagggagg gaggtaggta | cgtatgcgtg ctgctcagac caccgcaggg gaactggctg aacgtctgga tgctcatccc 840 | <pre><210 > SEQ ID NO 3 <211 > LENGTH: 1182 <212 > TYPE: DNA <213 > ORGANISM: Brevibacterium linens </pre> <pre><400 > SEQUENCE: 3 atgacctcgc tgcatcccga gacgctgatg gttcatggtg gaatgaaggg tctgaccgaa gctggagttc atgtcccggc aattgatctg agcaccacca atcccgtcaa tgatgtcgca accggtggag attcctatga gtggctggcc accggtcata cgctgaaaga tggtgatagc gcagtttatc agcgtctgtg gcagcctggg gttgctcgtt ttgaaaaccgc tctggcaggg ctggagcatg ctgaagaggc tgttgcattt gcaacgggta tggcagctat gactgcagct ctactggctg cagttcagc tggaaccacc cacattgttg ctgtaagacc tctgtacggt ggaagcgacc atctgctgga gaccggactg ctgggaacta cggttacttg ggctaaagag gcagatattg caagcgctat tcaagacgat accggactg ttatagttga aaccccggct aatccttcac tggatctagt cgacctggat tcggttgttt cggcagcagg taatgtaccc gtcctggtcg ataatacttt ttgtactccc gtcctgcagc agcctattc ccatggagct gcactggtcc tgcattctgc tactaagtat ctgggtggc acggtgacgc aatgggtggt attattgcaa ccaatgcaga ttgggctatg cgtctgagac aggttagagc aattaccgga</pre> | 60 120 180 240 300 360 420 540 600 660 720 |

| gcaatttccg | ttgttcatta | tccgggactg | aagggtcagg | atccccgtgg | actgctggga | 900 | | |
|------------|------------|------------|------------|------------|------------|------|--|--|
| cgtcaaatgt | ccgggggggg | ggcgatgatt | gctatggaac | tggcaggggg | ctttgatgca | 960 | | |
| gcacgtagtt | ttgttgagca | ttgtaatctg | gttgttcatg | ctgtatccct | gggtggtgct | 1020 | | |
| gatactctga | ttcagcaccc | ggcttcgctg | actcataggc | ccgtcgcggc | cacggcgaaa | 1080 | | |
| cctggtgacg | ggctgattag | actgtcggta | ggactggagc | atgttgacga | tctggctgat | 1140 | | |
| gacctgattg | ctgcactgga | tgettegegg | gcagctgcat | aa | | 1182 | | |

The invention claimed is:

- 1. A vector comprising a nucleic acid encoding a polypeptide comprising an amino acid sequence as set forth by SEQ ID NO: 1 or a functionally-conservative variant thereof.
- 2. The vector of claim 1, wherein the nucleic acid encoding for a polypeptide is selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 3.
- 3. A nucleic acid encoding a recombinant polypeptide comprising an amino acid sequence as set forth by SEQ ID NO: 1 or a functionally-conservative variant thereof.
- 4. The nucleic acid according to claim 3 comprising a sequence as set forth by SEQ ID NO: 2.
- 5. The nucleic acid according to claim 3 comprising a sequence as set forth by SEQ ID NO: 3.
 - 6. A host cell, which has been transformed by
 - a nucleic acid encoding a recombinant polypeptide comprising an amino acid sequence as set forth by SEQ ID 30 NO: 1 or a functionally-conservative variant thereof and/or
 - a vector comprising a nucleic acid encoding a polypeptide comprising an amino acid sequence as set forth by SEO ID NO: 1 or a functionally-conservative variant thereof.
 - 7. A pharmaceutical composition comprising
 - i) a recombinant polypeptide comprising an amino acid sequence as set forth by SEQ ID NO: 1 or a functionallyconservative variant thereof, or a nucleic acid encoding a polypeptide comprising an amino acid sequence as set forth by SEQ ID NO: 1 or a functionally-conservative variant thereof, or a vector comprising said nucleic acid,
 - ii) a pharmaceutically acceptable carrier.
- comprising one or more enzyme cofactors.
- 9. The pharmaceutical composition according to claim 7 comprising one or more chemotherapeutic or radiotherapeutic agents.
- 10. A recombinant polypeptide comprising an amino acid 50 sequence as set forth by SEQ ID NO: 1 or a functionallyconservative variant thereof contained in a vector.
- 11. A polypeptide comprising an amino acid sequence as set forth by SEQ ID NO: 1 or a functionally-conservative variant thereof which is covalently conjugated with at least 55 one polyethylene glycol group.

- 12. A polypeptide comprising an amino acid sequence as set forth by SEQ ID NO: 1 or a functionally-conservative variant thereof which is covalently coupled to a tumor target-
- 13. A method of producing a polypeptide comprising an amino acid sequence as set forth by SEQ ID NO: 1, which 20 method comprises the steps of:
 - i) culturing a host cell transformed by
 - a) a nucleic acid encoding a recombinant polypeptide comprising an amino acid sequence as set forth by SEQ ID NO: 1 or a functionally-conservative variant thereof,

- b) a vector comprising a nucleic acid encoding a polypeptide comprising an amino acid sequence as set forth by SEQ ID NO: 1 or a functionally-conservative variant thereof,
- under condition suitable to allow expression of said polypeptide; and
- (ii) recovering the expressed polypeptide.
- 14. A method for treating a cancer in a subject of need thereof comprising the step of administering to said subject a polypeptide comprising an amino acid sequence as set forth by SEQ ID NO: 1 or a functionally-conservative variant thereof.
- 15. A method for treating a cancer in a subject of need thereof comprising the step of administering to said subject a nucleic acid encoding a polypeptide comprising an amino acid sequence as set forth by SEO ID NO: 1 or a functionallyconservative variant thereof, or a vector comprising said nucleic acid.
- 16. A method for treating a cancer in a subject of need 8. The pharmaceutical composition according to claim 7 45 thereof comprising the step of administering to said subject a combination of
 - i) a polypeptide comprising an amino acid sequence as set forth by SEQ ID NO: 1 or a functionally-conservative variant thereof, or a nucleic acid encoding a polypeptide comprising an amino acid sequence as set forth by SEQ ID NO: 1 or a functionally-conservative variant thereof, or a vector comprising said nucleic acid, and
 - ii) one or more agents selected from the group consisting of: one or more enzyme cofactors and one or more chemotherapeutic or radiotherapeutic agents.